The striatum and the globus pallidus: Studies of internal and external organisation in the basal ganglia

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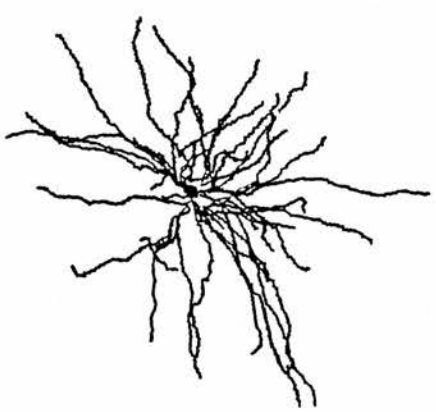
to Betty Krier,

with love and thanks



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Statement in terms of regulation 3.4.7

I declare that the studies presented in this thesis are the result of my own independent investigations, with the exception of the tracer injections and cell plotting involved in the neuroanatomy in Chapter 2 which were performed by Ann Wright.

Statement in terms of regulation 3.4.3: List of publications

Arbuthnott GW, Walker RH, Whale D, Wright AK (1983) Further evidence for a pallidostriatal pathway in rat brain. *J. Physiol.* 336:33P

Arbuthnott GW, Staines WA, Walker RH, Whale D, (1984) Pallidostriatal neurons with branches to the mesencephalon - electrophysiological evidence in the rat. *J. Physiol.* 346:33P

Walker RH, Graybiel AM, Baughman RW, Arbuthnott GW, (1988) A novel method for targeting neurons in a lightly fixed striatal slice preparation. *Soc. Neurosci. Abstr.* 14:1160

Walker RH, Arbuthnott GW, Wright AK, (1989) Electrophysiological and anatomical observations concerning the pallidostriatal pathway in the rat. *Exp. Brain Res.* 74:303-310

Walker RH, Arbuthnott GW, Graybiel AM, (1991) Intracellular labelling of medium spiny neurons in the primate caudate nucleus: Anatomical relationship of dendrites to striosomal borders. *Soc. Neurosci. Abstr.* 17:456

Abstract of thesis

The basal ganglia are crucial for the processing of motor commands received from the cerebral cortex, as is demonstrated by the disorders which follow lesions of this area. For example in humans, Parkinson's disease and Huntington's chorea, which are hypo- and hyperkinetic disorders respectively, arise from degeneration of two intimately connected structures within the basal ganglia, the substantia nigra and the neostriatum.

Increasingly, reciprocal connections between different nuclei have been described, suggesting that feedback loops are an integral part of this system. The globus pallidus receives most of its input from the striatum, and the medial part (the entopeduncular nucleus in lower species) is a major source of outflow from the basal ganglia. The afferent and efferent pathways of these nuclei are described. The first part of this thesis describes the anatomical and electrophysiological demonstration of a pathway from the globus pallidus in the rat to the neostriatum.

The second part of the thesis discusses the cellular level of organisation of the neostriatum. The discovery of histochemical compartmentalisation in the striatum, called striosomes and matrix, has suggested an anatomical division of function, corresponding approximately to sensory-motor and limbic functions. The vast majority of cells in the striatum are of a single morphological type, medium sized densely spiny neurons, which receive most of the input, and are the source of output from the striatum. The second part of the thesis describes the development of a technique which has permitted the study of the relationship of the dendrites of these cells to the borders between compartments. Although the striosome/matrix division is defined histochemically, it was found that dendrites of medium spiny cells did not necessarily observe borders. This finding has implications in terms of the roles of these cells in integrating information from the two compartments. The dendritic trees of medium spiny cells were found to have a marked orientation which paralleled the orientation of the striosomes. It is likely that this feature reflects an anatomical and functional relationship between the afferent and efferent connections of the striatum.

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6-OHDA	6-hydroxydopamine
AChE	acetylcholinesterase
BChE	butyrylcholinesterase
CaBP	calcium binding protein
CCK	cholecystokinin
ChAT	choline acetyltransferase
CM-pf	centre median, para fascicularis
DAB	diaminobenzidine
EM	electron microscope
EP	entopeduncular nucleus
GABA	gamma-aminobutyric acid
GAD	glutamic acid decarboxylase
GP	globus pallidus
HD	Huntington's disease
HRP	horseradish peroxidase
L-dopa	levo-dihydroxyphenylalanine
LGP	globus pallidus, lateral segment
LY	lucifer yellow
MAO	monoamine oxidase
MGP	globus pallidus, medial segment
MPDP+	1-methyl-4-phenyl-2,3-dihydropyridinium
m-phd	meta-phenylene diamine
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NBM	nucleus basalis of Meynert
NGS	normal goat serum
NMS	normal monkey serum
NOS	nitric oxide synthase
NPY	neuropeptide Y
PD	Parkinson's disease
SN	substantia nigra
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticularis
STN	subthalamic nucleus
VA	ventroanterior nucleus of thalamus
VL	ventrolateral nucleus of thalamus
VM	ventromedial nucleus of thalamus
VTA	ventral tegmental area

Chapter 1

Pathophysiology of the basal ganglia

Introduction

The basal ganglia consists of a group of nuclei situated in the midbrain, deep to the cortex. This term usually includes the neostriatum (formed by the caudate nucleus and putamen in higher mammals), the globus pallidus, the substantia nigra, and the subthalamic nucleus. Many connections of the various areas of the cortex with the peripheral nervous system pass through this region. For some pathways, cells from the basal ganglia form a link in the neuronal chain from cortex to peripheral nervous system; in other cases fibre bundles pass straight through, for example, those forming the internal capsule. The neuronal signals carried along these pathways can be influenced at many sites by input from other areas of the brain. There appear to be many reciprocal connections between the different nuclei of the basal ganglia and other subcortical structures implying that feedback regulation is important in their functioning (figure 1.1).

The cortex is generally understood to be responsible for the higher functions of the human brain; it is also the origin of motor commands and the site of processing of sensory signals. Much is known about the cellular mechanisms of cortical function. The functions of the various nuclei of the basal ganglia, however, are far from obvious. Their strategic situation implies that they are important for processing neuronal information

originating in the cortex. Evidence from experimental lesions in animals and from neuropathological data, for example, in Parkinson's and Huntington's diseases, tells us that they are essential for the control of motor function.

The loss of cells from the substantia nigra produces the commonest disorder of movement, Parkinson's disease, a major symptom of which is a limitation of movement - hypokinesia. This contrasts with lesions of other parts of the basal ganglia, which result in different types of hyperkinesia.

Substantia Nigra - Parkinson's Disease

Clinical

In 1817 James Parkinson first described the disease which is named after him in "An Essay on the Shaking Palsy" (Parkinson, 1817). He described a disorder characterised by a fine "pill-rolling" tremor of the hands at rest at a frequency of 3-7Hz, slowness of movement or bradykinesia, difficulty of initiating movement (akinesia), reduction in amplitude of voluntary and involuntary movements (hypokinesia), rigidity (hypertonia). There is an increase in tone of the axial muscles, causing a stooping posture with loss of postural reflexes. Other symptoms and signs are a paucity of involuntary movements such as swinging the

arms when walking, and cogwheel and leadpipe rigidity of the limbs.

There are many other characteristic signs of this illness, such as a decrease in the size of the person's handwriting (micrographia). The typical gait is described as "festinating" or hurrying, where the steps are small and fast (marche a petits pas), with an element of uncontrolled acceleration, exacerbated by the stooping posture. The affected person seems to become progressively "locked in", with an expressionless "mask-like" facies (hypomimea) and loss of volume and modulation of speech (hypophonia).

Other signs and symptoms occur which are not related to the motor system. Personality changes can develop, distinct from the depression which might be expected in response to this debilitating illness. There can be peripheral sensory symptoms, and the reappearance of primitive reflexes, such as the glabellar tap reflex, which would suggest some sort of brainstem disinhibitory process.

Environmental stimuli can greatly affect the extent of the expression of the disorder. People who are in the early stages of the disease can experience "freezing" in response to stressful stimuli. However, there are also particular types of stimuli which can facilitate normal movement in affected individuals. People with

Parkinson's disease often have no problem climbing stairs, and they find walking on a level surface easier if there are lines for them to step over. There is the classic anecdote about the nursing home on fire where the population of heretofore immobile parkinsonian patients spring to life in the presence of a life-threatening emergency (kinesia paradoxica).

This progressive disorder can start as young as thirty, with incidence increasing with age, and occurs in one person in one thousand (Pollock and Hornabrook, 1966), affecting men more than women with the ratio 3:2. The course of the disease can vary, but usually follows a pattern of increasing limitation of movement (Hoehn and Yahr, 1967), with a mortality of three times that of the general population (Pollock and Hornabrook, 1966). Various schemes have been developed to describe the clinical progress of the disease; most commonly used is the Hoehn and Yahr classification which relates to the level of motor dysfunction (Hoehn and Yahr, 1967). The commonest cause of death in Parkinson's disease is pneumonia, when the lack of mobility, especially of involuntary movements means that the lungs cannot be cleared of secretions and infection sets in. A frequent cause of morbidity is falls (Koller *et al.*1989) for which Parkinson's disease (PD) significantly increases the risk (Nevitt *et al.*1989). This appears to be mainly due to the loss of postural reflexes but is also

exacerbated by the fact that affected individuals cannot move fast enough to correct their balance (Nevitt *et al.*1989). For similar reasons they also often suffer from bedsores. Urinary tract infections are a problem as they are in many who are chronically immobile.

Pathology

Post mortem examination of the brains from affected patients reveals a loss of over 90% of the cells of the substantia nigra pars compacta (SNc). These are marked in unaffected human brain by a deposition of dark pigment for which the substantia nigra is named (Hornykiewicz, 1966). The dark pigment, neuromelanin, is a by-product of dopamine synthesis, the neurotransmitter used by the cells of the nigrostriatal pathway (Anden *et al.*1964; Dahlstrom and Fuxe, 1964).

There is a large decrease in levels of tyrosine hydroxylase (TH), the synthetic enzyme for dopamine (McGeer and McGeer, 1976). These workers also found decreases in levels of glutamic acid decarboxylase (GAD), the synthetic enzyme for gamma-aminobutyric acid (GABA), in the globus pallidus (GP) and substantia nigra (SN) (McGeer and McGeer, 1976), although Perry *et al.*(1983) found that striatal GABAergic activity was increased.

The striatal cells seem to compensate for loss of dopaminergic input by upregulation of dopamine receptors

(Lee *et al.*1978; Guttman and Seeman, 1985). Other authors (Rinne *et al.*1991), however, using binding techniques in human tissue, saw only a small decrease in D2 receptors, regardless of duration of disease or L-dopa treatment. Other mechanisms, such as an increase in cell activity, may also have a role in functionally compensating for the defect. Symptoms only appear after dopamine is depleted by more than 90%.

The putamen is affected more than the caudate (Bernheimer *et al.*1973 cited in Hornykiewicz, 1979a; Kish *et al.*1988), which correlates with its higher involvement with motor function. There is also a loss of noradrenaline in the locus coeruleus, nucleus accumbens and hypothalamus (Farley and Hornykiewicz, 1976). A feature which is not specific, but which is usually regarded as being essential for a neuropathological diagnosis of PD, is the presence of Lewy bodies, which are eosinophilic inclusions with a concentric halo in the cytoplasm of cells of the substantia nigra and the locus coeruleus.

Aetiology

The cause of Parkinson's Disease is not known. There is no evidence from twin studies (Ward *et al.*1983) that it is has a major genetic component. The influenza epidemic of 1918 caused a long term sequela of encephalitis lethargica (Von Economo's encephalitis),

which was characterised by a parkinsonian disorder. This syndrome was remarkably responsive to levo-dihydroxyphenylalanine (L-dopa) therapy when this was discovered in the 1960's, but it has remained a distinct clinical entity without connection to idiopathic Parkinson's disease. With the discovery of the neurotoxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (see below) there was support for the hypothesis of a widespread environmental toxin which causes PD (Lewin, 1985; Langston, 1987), but to date no single causative agent has been identified.

MPTP

In 1979 (Davis *et al.*) the first case was described of an injecting-drug user who had developed a parkinsonian syndrome following injection of a synthetic opiate. In 1982 (Langston *et al.*1983; Ballard *et al.*1985) a number of users of street drugs in Northern California were affected by a disorder that appeared to be clinically identical to a severe and accelerated form of Parkinson's disease, and more research was stimulated. These patients responded well to L-dopa, but with identical complications to those seen in idiopathic PD (Ballard *et al.*1985) in that many of them quickly developed severe on-off effects. In the only case to come to *post mortem* so far (Davis *et al.*1979) there was loss of the pigmented cells of the substantia nigra with Lewy bodies present, and minimal changes of the locus coeruleus.

There have also been other reports of MPTP toxicity following casual exposure of a chemist (Langston and Ballard, 1983).

Langston and co-workers (1983) discovered that the chemical process used to make meperidine, a synthetic equivalent of heroin produced a substance known as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Ziering *et al.*1947). A metabolite of this, MPP+, specifically destroys the dopaminergic nigrostriatal cells (Burns *et al.*1983; Langston *et al.*1984; Irwin and Langston, 1985) by binding to neuromelanin (D'Amato *et al.*1987).

MPTP passes through the blood-brain barrier in its uncharged state, and is then oxidised, maybe in astrocytes (Ransom *et al.*1987), by monoamine oxidase B to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+), and then to 1-methyl-4-phenylpyridinium (MPP+). This is taken up selectively by the dopamine uptake system (Ricuarte *et al.*1985) into the nigrostriatal cells. Here it binds to neuromelanin (D'Amato *et al.*1987) and somehow causes the cytotoxic damage with loss of cells and of pigment in the SNc (Langston *et al.*1984; Hirsch *et al.*1988).

The data from humans is limited at present, and seems to

suggest that the lesion is more specific, damaging mainly the nigrostriatal cells, than the lesion seen in idiopathic PD, although clinically it appears identical. It affects the caudate nucleus and the putamen equally, and in this aspect resembles post-encephalitic parkinsonism. Animal data (see below) has demonstrated, however, that the damage produced by MPTP may be more widespread.

Treatment

In 1961 (Birkmayer and Hornykiewicz, 1961; Birkmayer and Hornykiewicz, 1962, cited in Birkmayer, 1979; Cotzias *et al.*1967; Yahr *et al.*1969) it was discovered that the crude replacement of the neurotransmitter dopamine by the prescription of L-dopa (levo-dihydroxyphenylalanine) the precursor of dopamine, could ameliorate some of the symptoms of this crippling motor disorder.

Dopamine itself is charged and does not cross the blood-brain barrier. L-dopa can cross into the central nervous system, and is converted to dopamine by endogenous dopa-decarboxylase (aromatic amino acid decarboxylase). L-dopa is taken orally, along with an inhibitor of dopa-decarboxylase (which does not cross the blood-brain barrier) to prevent the reaction happening in the peripheral tissues. Until the addition of a peripheral decarboxylase inhibitor, the doses of L-dopa necessary to achieve an alleviation of symptoms produced major peripheral side-effects (Yahr *et*

*al.*1969).

Anticholinergic drugs, such as benztropine, diphenhydramine, ethopropazine, trihexiphenidyl, and artane have been used in the treatment of Parkinson's disease long before the discovery of L-dopa. These drugs appear to ameliorate specifically the tremor and rigidity, and in concert with dopaminergic agents can be very effective in replacing the actions of the lost cells. On a crude level there appears to be a functional balance within the striatum between complimentary cholinergic and dopaminergic actions.

More recently, dopaminergic agonists such as bromocriptine, and L-deprenyl (Selegiline) which inhibits monoamine oxidase B, a degradative enzyme of dopamine (Green *et al.*1977), have been used with success (Birkmayer *et al.*1977). Thus these drugs can potentiate dopaminergic transmission, by preventing the breakdown of the remaining dopamine.

There is some evidence that administration of deprenyl in combination with L-dopa to PD patients in the early stages of the disease can slow down the rate of progression and increase life expectancy (Birkmayer *et al.*1985; Shoulson, 1989; Tetrud and Langston, 1989). MPTP is converted to its neurotoxic form by MAO-B, which can be inhibited by deprenyl (Heikkila *et al.*1985), and

this implicates activity of MAO-B in the development of idiopathic PD.

The antiviral agent amantidine hydrochloride is sometimes effective in the early stages and it acts by blocking re-uptake.

The usefulness of these pharmaceutical agents, however, is often limited to relatively early stages of the disease, and after a few years patients often start to experience various side-effects. At the peak of the dose effect, there may be effects of apparent overstimulation of dopamine receptors, resulting in dyskinesia. This may be helped by alteration of the dosage regime to lower doses more frequently. However, later there may develop what is called the "on-off" phenomenon; their symptoms fluctuate wildly, a phenonemon which is only partially related to blood levels of drugs (Marsden, 1990).

Some trials have found that intravenous or subcutaneous infusions of the dopamine D2 agonist lisuride or L-dopa (Hardie *et al.*1984; Quinn *et al.*1984; Obeso *et al.*1986) ameliorate fluctuations, by providing a more continuous stimulation of dopamine receptors. However, a practical clinical solution to the problem has not been found.

The role of dopamine in the striatum

S.A.K. Wilson believed in 1925 that the striatum was the site of the pathology of Parkinson's disease (1925a; 1925b; 1925c; 1925d). Although we now know that the cells lost are those of the substantia nigra, the effects of their absence are felt in the striatum. How does the loss of this single pathway consisting of (apparently) a single cell type and a single transmitter explain the variety of signs and symptoms?

There has been apparently conflicting evidence about the role of dopamine in the striatum. The best way to interpret this may be say that its actions are determined by many parameters, both pre- and post-synaptic.

Dopamine receptors

Until relatively recently there were two known types of dopamine receptors (Kebabian and Calne, 1979; Creese *et al.*1983; Stoof and Kebabian, 1984; Clark and White, 1987). Despite extensive investigation their precise physiological roles have not been fully chraracterised. The conflicting evidence as to their actions may be explicable by the recent discovery of a number of other subtypes of dopamine receptors.

The D1 receptor, which has been characterised genetically (Dearry *et al.*1990; Monsma *et al.*1990;

Sunahara *et al.*1990; Zhou *et al.*1990), stimulates adenylate cyclase and cyclic AMP-dependent protein kinases, and is also coupled to phospholipase C and calcium mobilisation. This receptor seems to function usually as the postsynaptic target of dopamine. It can induce certain kinds of stereotyped behaviour, such as grooming in rats. However the lack of a drug with selective D1 effects (Kebabian *et al.*1986; Waddington, 1988) has made the precise characterisation of its role difficult. In the striatum, D1 receptor mRNA is located predominantly in the substance P-containing cells which project to the medial globus pallidus (MGP) and substantia nigra pars reticulata (SNr), with some cells also expressing D2 receptor mRNA(Gerfen *et al.*1990; Le Moine *et al.*1991). Le Moine and co-workers (1991) also found that the cholinergic interneurons had a weak signal for D1 receptor mRNA. A second type of D1 receptor, D1B, has recently been cloned (Tiberi *et al.*1991). It has a distinctly different distribution from the previously described D1 receptor, and shows no binding in the striatum. D1 receptors do not seem to reflect chronic changes in dopaminergic input in the same way that D2 receptors do, and show a slight decrease by receptor autoradiography in the rat model of PD, the 6-hydroxydopamine (6-OHDA)-lesioned rat (see below) (Joyce, 1991).

The D2 receptor has a functionally opposite effect in that it inhibits adenylate cyclase, or stimulates guanine

triphosphate (GTP). It can be found presynaptically, as well as postsynaptically, and is assumed to be responsible for negative feedback. It has 2 isoforms A and B. There is much indirect (for example, Walters *et al.*1987)) and less direct (Seeman *et al.*1989), evidence that the two receptors, D1 and D2, are closely linked functionally, possibly by an intracellular second messenger system (Seeman *et al.*1989). Using a probe for the mRNA for the D2 receptor, it appears to be primarily localised to the medium-sized enkephalin-containing neurons which project to the lateral globus pallidus (LGP) (Gerfen *et al.*1990; Le Moine *et al.*1990a; Le Moine *et al.*1991). MRNA for the D2 receptor was also found in large cells, probably the cholinergic interneurons. Administration of haloperidol, a nonspecific dopamine antagonist, increased the amount of D2 mRNA expressed in medium sized cells, and the number of large cells which contain it (Gerfen *et al.*1990; Le Moine *et al.*1990a; Le Moine *et al.*1990b; Le Moine *et al.*1991). This is consistent with the increase in D2 receptors after haloperidol treatment. This increase is also seen, using receptor autoradiography (Joyce, 1991) in the 6-OHDA-lesioned rat.

The D3 receptor (Sokoloff *et al.*1990), is found both pre- and postsynaptically, and appears from its distribution to be associated with limbic functions.

the D2 and D3 receptors, but has a much higher affinity for the antipsychotic drug clozapine and is distinguished by being localised to frontal cortex, midbrain, amygdala and medulla, with far lower levels in the basal ganglia.

The D5 receptor (Sunahara *et al.*1991) appears to be more like the D1 receptor in its structure and activity, but with a much higher affinity for dopamine itself.

Most experiments on the electrophysiological and behavioural effects of different dopaminergic agents have interpreted results in terms of D1 or D2 activation, but the recent characterisation of other receptors, and of D1 and D2 subtypes, may enable more accurate descriptions in terms of a balance of D1/D5 and D2/D3/D4 interaction.

Actions of dopamine in the striatum

Williams and Millar (1990) measured dopamine release produced by stimulation of the medial forebrain bundle *in vivo* in rats, using a form of electrochemical detection. They described varying effects upon the activity of striatal cells, but often saw excitation. When the amount of dopamine present rose, however, the cells were usually inhibited, suggesting a concentration-dependent effect. Some cells were inhibited at all levels and could be interneurons with a

single response to dopamine.

In intracellular studies in the rat *in vitro* slice (Calabresi *et al.*1987) the effect of dopamine upon striatal (presumably medium-sized densely spiny) cells was inhibitory. The threshold for firing spikes was raised by a mechanism that was blocked by a D1 dopamine receptor antagonist SCH 23390, and effected by a D1 dopamine receptor agonist SKF 38393 (Calabresi *et al.*1987; Akaike *et al.*1987). This appeared to be due to a decrease in the voltage-dependent sodium current (Calabresi *et al.*1987; Surmeier *et al.*1991). Thus more depolarisation was required to generate an action potential.

Brown and Arbuthnott (1983) observed an effect of dopamine *in vivo* which reduced the spontaneous activity, and reduced the excitatory effect of cortical stimulation upon striatal cells. This was due to an action upon D2 receptors.

Rutherford and co-workers (1988), using a similar preparation, described, in addition to the inhibitory effect of raising the threshold, an afterhyperpolarisation of striatal cells which was reduced in size by the application of dopamine. This effect is excitatory, as it would permit more frequent generation of action potentials. This also appeared to

be due to an action upon D2 receptors (Akaike *et al.*1987).

Surmeier and co-workers (1991) found D2 agonists to produce opposing effects upon different cells, and suggested that one of these might be due to an action upon D3 receptors.

There has been debate as to whether D1 and D2 receptors are located upon the same striatal cells. Gerfen and co-workers (1990) find that the populations are primarily separate, with colocalisation in a relatively small percentage of neurons.

Synergism by the two receptor types has recently been demonstrated *in vitro*. Bertorello and co-workers (1990) have shown synergistic inhibition of the sodium/potassium ATPase pump. Piomelli and co-workers (1991) demonstrated a synergism in releasing arachidonic acid, which is extensively involved in second messenger systems. These studies provide evidence that the two receptors can interact on a molecular level, and suggest that this might be the case *in vivo*.

The function of dopamine in the striatum can be one of either inhibition or excitation, depending upon the amount of dopamine present and substrate of its action. This is the cause of the functionally complex effects of its deficiency.

Animal models of Parkinson's disease

Rat

The nigrostriatal tract can be specifically lesioned in rats by administration of 6-hydroxydopamine (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1968). Bilateral lesions make the animals aphasic and adipsic and are often fatal, but unilateral injections cause contralateral motor signs. The animal's posture is such that it turns towards the lesioned side. There is a denervation supersensitivity of striatal dopamine receptors (Creese *et al.*1977), as demonstrated by the effect of dopaminergic agonists which cause the animal to rotate away from the lesioned side (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1968). Zigmond and Stricker (1984) found that many characteristics of these lesioned rats reproduced those of parkinsonian patients, and explained them in terms of neurochemistry. For example, the stress-induced deterioration of mildly affected early PD patients could be due to an acute depletion of releasable dopamine, in a system which has been previously compensating by functioning at a higher level of activity but with less reserve capacity.

Rats are not very susceptible to the dopamine-depleting effects of MPTP, presumably because they do not have neuromelanin in the substantia nigra. However, administering intensive dosing regimes to older animals

has resulted in losses of striatal dopamine (Jarvis and Wagner, 1990).

Primate

Initially investigators tried to reproduce the disorders of PD by performing stereotaxic lesions of the nigrostriatal tract in monkeys (Poirier and Sourkes, 1965; Sourkes and Poirier, 1966; Stern, 1966; Poirier *et al.*1966). Although the defect produced was similar in many ways to the human syndrome, this lesion was not specific for the dopaminergic neurons.

It was hoped that discovery of MPTP-induced PD (see above) would shed light upon the cause of Parkinson's disease, and that this would lead to the discovery of an accurate animal model of Parkinson's disease. This hope has been partially fulfilled, but has not yielded as many answers as was initially hoped. Administration of MPTP to primates (Burns *et al.*1983; Langston *et al.*1984), produces a disorder of movement which is identical to PD in many ways, responding similarly to L-dopa therapy, and with similar problems (Chiueh *et al.*1985; Schultz *et al.*1985; Schultz *et al.*1989; Porrino *et al.*1987).

In primates treated with MPTP, and examined histologically, there was loss of the dopaminergic cells of the substantia nigra pars compacta, with swollen ascending dopaminergic axons (Kitt *et al.*1986). This

loss of dopaminergic input is reflected by a loss of 3[H]-mazindol binding sites (Joyce *et al.*1985) which label dopamine-uptake sites on presynaptic terminals. As in PD loss of at least 90% of these cells was necessary for expression of the motor disorder. An upregulation of D2 receptors in the dorsolateral putamen (Joyce *et al.*1985) is seen as a compensatory mechanism. Animal experiments, in contrast to data from humans, have shown damage to the mesolimbic dopaminergic cells of the ventral tegmental area (VTA) and the locus coeruleus (Mitchell *et al.*1985; Di Paolo *et al.*1986; Schneider *et al.*1988; German *et al.*1988; Pifl *et al.*1991). Namura and co-workers (1987) demonstrated that MPTP could be toxic to noradrenergic and serotoninergic, as well to dopaminergic neurons in the rat, but only after direct intracranial injection.

Parkinson's disease is characterised by the presence of eosinophilic inclusions called Lewy bodies. Similar, but not identical structures have been seen in aged primates treated with MPTP (Forno *et al.*1988). The authors speculate that these inclusions could be an immature form of Lewy body.

Pifl and co-workers described dopamine to be lost equally after MPTP administration in both caudate and putamen. This is one aspect in which the MPTP-induced lesion resembles post-encephalitic parkinsonism rather than idiopathic PD (Pifl *et al.*1938; Pifl *et al.*1991).

Despite initial difficulties in reproducing the precise histological lesions of PD in MPTP-induced parkinsonism in primates, more recent data suggests that the lesions are very similar. The MPTP lesion also seems to duplicate the clinical picture of idiopathic PD, despite the minor anatomical discrepancies, and has been used extensively in studies of the mechanism of this disorder.

Mechanism of motor disorder: The role of the globus pallidus

A wide range of symptoms, which can be described as "positive", such as tremor and rigidity, and "negative", such as akinesia, bradykinesia, and hypokinesia, result from the loss of one group of cells of one anatomical type, apparently utilising one neurotransmitter. This question can be answered in part by studies of the activity of the globus pallidus, which is the recipient of the major efferent pathway from the striatum (figure 1.1).

This nucleus is made up of two parts, lateral or external and medial or internal, in the primate, with the latter equivalent in the rat to the entopeduncular nucleus (EP). It is directly related to movement. In experiments recording from conscious monkeys (Delong, 1971) pallidal cells fire when the animal makes an active

movement on the contralateral side. Changes in activity of pallidal cells in animal models of PD are intimately related to the effect of dopamine upon striatal cells. The medial and lateral GP both receive GABAergic afferents from the striatum, with the same cells containing one of the peptidergic neurotransmitters, substance P and met-enkephalin. There is increasing evidence that they receive inputs from separate populations of striatal cells, and are affected differentially by the loss of dopaminergic input to the striatum.

Bergstrom and co-workers (Bergstrom *et al.*1982; Bergstrom and Walters, 1984) found that dopamine increased the firing rate of rat GP cells, and that dopamine reduced the inhibitory effect of GABA upon GP cell firing rates. Toan and Schultz (1985) recorded in the rat GP following stimulation of the cortex, and observed the effect of stimulation of the substantia nigra and of neuroleptic drugs. They found that the effect of dopamine was to reduce the response of most pallidal cells to cortical stimulation. The effect of dopamine would be to block out all but the strongest stimulatory effects.

Evidence from primate models of Parkinson's disease (Filion *et al.*1986; Filion, 1979; Filion *et al.*1988; 1989; 1991) shows that the two segments of the globus pallidus react in opposite directions to a lesion of the

nigrostriatal system, the MGP cells becoming faster and more bursting and the LGP cells slower and less bursting.

Metabolic studies using the 2-deoxyglucose (2-DG) method (Sokoloff *et al.*1977) have been very useful in describing the relative activity of different pathways. An increase in labelling is believed to be due to an increase in the activity of the sodium-potassium ATPase pump in afferent synaptic terminals (Schwartz *et al.*1979; Mata *et al.*1980).

Studies in MPTP-treated primates (Crossman *et al.*1985; Mitchell *et al.*1986; Sambrook *et al.*1989; Palombo *et al.*1990), and in 6-OHDA lesioned rats (Wooten and Collins, 1983) demonstrated an increase in activity of striatal efferent pathways. There was increased labelling in both segments of the GP, especially in the lateral segment. This was reversible in the rat by the dopaminergic agonist apomorphine. The LGP projects predominantly to the subthalamic nucleus (STN), which would be disinhibited, with increased excitation of the MGP cells and more inhibition of the thalamus and the thalamocortical connection. The reverse of these results is seen in hyperkinetic disorders (see below).

Interestingly, a lesser increase in terminal activity is seen in the MGP. Electrophysiological changes in the MPTP primate are in the opposite direction in the MGP

(Filion *et al.*1989; 1991), and are more likely to be due to an increase in the excitatory STN-MGP pathway. This would suggest that the striato-MGP pathway is less significant than the input from the STN, but does not explain the small size of the increase with 2-DG labelling. The axons of striatopallidal neurons branch extensively in the GP, and maybe they contact the distal dendrites of MGP cells, whilst STN axons contact the cell bodies and thus would be more effective.

Another interpretation is that there are differential actions of dopamine upon striatal cells projecting to different sites. Robertson and co-workers (1989) looked at GABA receptor binding in MPTP-treated primates, and found that the level decreased in the LGP, and increased in the MGP. This they explain in terms of compensating for the altered striatopallidal activity - an increased striatal input to LGP, and decreased to MGP. Robertson and co-workers (1989) suggest that rather than being a differential action in the striatum, it is due to an action of dopamine in the MGP, acting presynaptically upon striato-MGP terminals. Parent and Smith (1987) have described a dopaminergic projection from the SNc in primates to pallidum, predominantly medial GP, and to a lesser extent to LGP. Robertson and co-workers (1989) suggest that under normal circumstances the presynaptic D1 receptors, (located primarily in MGP; Graham and Crossman, 1987; Beckstead *et al.*1988) are necessary for

release of GABA from striatopallidal terminals. In the MPTP-treated animal and in PD this activation would be absent, and binding for GABA receptors goes up. However, Schneider and Dacko (1991) have shown that the dopaminergic nigropallidal input is spared in MPTP-treated primates.

Bergman and co-workers (1990) lesioned the STN in MPTP-parkinsonian monkeys, and alleviated three major parkinsonian components, akinesia, tremor and hypokinesia. Lesioning the STN would remove the overactive inhibitory projection from the MGP to the ventroanterior/ventrolateral (VA/VL) thalamus. The dyskinesia which would be expected following damage to the STN was only a temporary problem in this study. This supports a critical role of the STN in regulating basal ganglia outflow via the MGP.

Iatrogenic PD and Tardive dyskinesia

The neuroleptic drugs used to treat schizophrenia - phenothiazines such as chlorpromazine and thorazine, and butyrophenones such as haloperidol, are dopamine antagonists. Chronic use and particularly abuse of these drugs can result in iatrogenic Parkinson's disease, due to antagonism of dopamine to an extent which mimics the loss of dopamine due to degeneration of the nigrostriatal pathway. The most effective treatment is to stop the causative drugs if possible. The

different effects - motor or psychiatric - of different dopaminergic agonists and antagonists has been partially explained in terms of actions at different dopamine receptors, D1 or D2. To date this has not been very satisfactory, but the identification of the D3, D4 and D5, dopamine receptors, with different affinities and locations may help explain these differential effects.

Tardive dyskinesia is also a symptom of long-term neuroleptic use. It may be due to receptor supersensitivity, in which the response of postsynaptic cells to chronic blockade of dopamine receptors (List and Seeman, 1979; Reches *et al.*1982), is to increase the number of D2 receptors (but see Waddington and co-workers, 1989). The resulting involuntary, rhythmic, repetitive writhing movements of the orolinguobuccal muscles, and also of the trunk are reminiscent of athetoid movements.

Symptoms can be relieved by reserpine, which depletes nerve terminals, and by alpha-methyl-paratyrosine which interferes with catecholamine synthesis, although these are not practical clinically, as the resulting neurotransmitter deficit can cause parkinsonism, and other severe side-effects.

Other causes of Parkinsonism

Parkinsonian syndromes can be produced by various other

causes, such as hypoparathyroidism or poisoning by manganese or cobalt, which damage the basal ganglia. These disorders tend to respond well to anticholinergic drugs, suggesting that the mechanism of the defect is somewhat different from in more specific nigrostriatal degeneration. As with many intracerebral lesions, the same result can be caused by cerebrovascular disease, where multiple infarcts can damage the nigrostriatal tracts bilaterally.

Parkinsonism can be a part of the syndromes of other neurological illnesses, such as striatonigral degeneration, progressive supranuclear palsy, Wilson's disease (see below), Alzheimer's disease, Huntington's disease, Shy-Drager syndrome (orthostatic hypotension), normal pressure hydrocephalus, olivopontocerebellar degeneration (cerebellar ataxia) and others.

Neostriatum - Huntington's Disease

Clinical

In 1872, George Huntington described a disease (cited in Martin, 1984) which has now been characterised to the level of a genetic marker (Gusella *et al.*1983). Huntington's disease is an autosomal-dominantly inherited disease, with almost 100% penetrance, and a prevalence of 4-7/100,000 in European populations (lower prevalence in people of African or Asian descent) (Schoenberg, 1979). Almost all cases have been traced to ancestors living in Europe, suggesting that it is rarely

due to spontaneous mutations (Martin, 1984). Apart from the well-known Long Island, New York, cases described by Huntington, hereditary chorea was described in Norway, Lancashire, and other places in the late 1800's (Bruyn, 1968; Martin, 1984).

This disorder is characterised by the onset, usually between twenty-five and fifty years of age (McDowell and Cedarbaum, 1987) although sometimes younger, of uncontrollable unpredictable flailing of the limbs, described as chorea (from the Greek chorea=dancing).

The duration is usually of between 12-16 years (Kurtzke, 1979). This motor dysfunction is invariably accompanied by a progressive psychiatric disorder (Paulson, 1979; McDowell and Cedarbaum, 1987) which may vary in its manifestation. Initial symptoms may be mild; fidgetting, irritability and absentmindedness, progressing to depression, drowsiness and falls, with a general depression of mental functioning. Speech may become slurred, in combination with facial dyskinesia. Sometimes there may be a more defined psychiatric condition, such as depression, bipolar affective disorder, schizophreniform symptoms with auditory hallucinations and/or dementia (McDowell and Cedarbaum, 1987).

Pathology

Upon post mortem examination of the brains of affected

individuals, there are anatomical and biochemical changes in a number of areas in the brain corresponding to the severity of the illness. In particular there is atrophy of the striatum, predominantly of the caudate nucleus (Bruyn, 1968; Vonsattel *et al.*1985), often to the extent that the wall of the lateral ventricle is no longer indented by the head of the caudate nucleus, but instead is convex laterally. Areas closely related to the striatum are also affected, such as the cerebral cortex (by as much as 20%) and globus pallidus, and there are signs of degeneration in many other parts of the brain, such as the dentate nucleus and inferior olive of the cerebellum (Bruyn *et al.*1979).

The destruction of the caudate is not uniform, and affects only certain cell types, with the concomitant decrease of some neurotransmitters more than others. Cells are lost from the area which has been characterised as extrastriosomal matrix (Ferrante *et al.*1986; Feigenbaum *et al.*1986; Joyce *et al.*1989), as would be expected as this compartment is associated predominantly with motor function.

The most striking biochemical change is a large drop in the amount of GABA in the caudate nucleus-putamen (Perry *et al.*1973; Ellison *et al.*1987) and its synthetic enzyme, glutamic acid decarboxylase (GAD) (Bird and Iversen, 1974; McGeer and McGeer, 1976; Spokes, 1980) compared to controls. This reflects the striking loss

of projection neurons and is also seen in the target sites (Spokes, 1980; Ellison *et al.*1987). The first losses are seen in the projection to the LGP (Bruyn, 1968; Ellison *et al.*1987; Reiner *et al.*1988), with a loss of enkephalin in the striatum (Ferrante *et al.*1986) and in the LGP. These changes are seen at a later stage with the projection to the sustantia nigra pars reticulata (SNr) (Emson *et al.*1980; Reiner *et al.*1988). There is a drop in levels of substance P in the striatum (Ferrante *et al.*1986), the substantia nigra pars reticulata (Kanazawa *et al.*1977; Emson *et al.*1980; Aronin *et al.*1983; Reiner *et al.*1988) and both parts of the GP (Emson *et al.*1980; Aronin *et al.*1983). Graveland and co-workers (1985a) found striking abnormalities of the morphology of the medium-sized densely-spiny cells, which represent the vast majority of the striatal cell population, with variously loss or overgrowth of spines, and recurvature of the tips of dendrites, suggestive of regrowth. The anatomical distribution of these changes followed the grosser level pathology.

The caudate nucleus and putamen are divided into two histochemically-defined compartments (Graybiel and Ragsdale, 1978), a system of interconnecting islands - striosomes - set in a background matrix. These two areas are differentially affected in HD. The relative preservation of D2 binding sites and 3[H]-mazindol labelling (Joyce *et al.*1989) supports the idea that the

dopaminergic input to the matrix is intact. There was a marked loss, most pronounced dorsolaterally, of D1 binding sites (Joyce *et al.*1989) which are located upon medium spiny neurons.

A decrease is seen in the levels of acetylcholine and choline acetyltransferase in the Huntington's disease (HD) striatum (Spokes, 1980; Bird and Iversen, 1974), although there does appear to be preservation of the acetylcholinesterase (AChE) staining of the matrix, with evidence that the large cholinergic interneurons themselves are spared (Ferrante *et al.*1986; 1987). There is not a relative increase of tyrosine hydroxylase staining (Ferrante and Kowall, 1987), suggesting that the dopaminergic input to the striatum decreases in proportion to the striatal atrophy. Bird and Iversen (1974) found no change in levels of dopamine, although in cases which they classified as "rigid", there was a significant increase in dopamine levels. Spokes (1980) found an increase of approximately 50% in tissue dopamine levels in HD, this may have been due to a selection of particularly severely affected patients. The loss of the cortically-projecting nucleus basalis of Meynert (NBM) cholinergic cells is probably related to the appearance of dementia as part of the illness.

The medium-sized aspiny interneurons containing neuropeptide Y (NPY), somatostatin, and NADPH-diaphorase are spared (Ferrante *et al.*1985) thus leading to a

relative increase in tissue levels, due to tissue destruction (Aronin *et al.*1983). There is some evidence that levels of these substances are abnormally increased with the formation of abnormal neuronal structures (Beal *et al.*1985; Marshall and Landis, 1985).

Aetiology

The characteristics of the neuronal damage seen in HD are widely believed to indicate excessive production of an endogenous excitotoxin, despite the fact that the lesion is yet to be precisely replicated using known excitotoxins in an animal model.

It has been demonstrated recently that the excitotoxic effect of glutamate upon cortical cell cultures is mediated by nitric oxide (NO) (Dawson *et al.*1991b). Nitric oxide synthase (NOS) is localised in the NPY/somatostatin cells (Bredt *et al.*1991) and has in fact been identified as NADPH-diaphorase (Dawson *et al.*1991a). As these cells survive in HD, it may be that NOS is a protective factor and that NO is the source of toxicity to surrounding cells (Dawson *et al.*1991b).

Animal models

An animal model of Huntington's disease has been very difficult to develop. A non-specific destructive lesion of the striatum does not reliably cause chorea (Wilson, 1914; Kennard, 1944). Excitotoxin lesions have been

shown to destroy specific cell populations, but the problem of replicating this dyskinesia in animals, and the failure of neurotransmitter replacement to correct it in humans, has suggested both a more specific pathogenesis and a more sophisticated role for striatal GABAergic neurons.

Injections of various excitotoxins such as quinolinic acid (Schwarcz *et al.*1983; Schwarcz *et al.*1984; Beal *et al.*1986; Ellison *et al.*1987), ibotinic acid, kainic acid (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976; Schwarcz and Coyle, 1977; Beal *et al.*1985) or glutamate itself (McGeer and McGeer, 1976; McBean and Roberts, 1984) have replicated to certain extents to biochemical lesions in rats, but a primate model has not been developed. The specificity of the cell loss seems hard to duplicate.

Beal and co-workers (1986) found that quinolinic acid injections into rat striatum more accurately mimic the cell loss seen in HD. Davies and Roberts (1987), however, saw no selective preservation after injection of quinolinic acid. They documented a loss of NADPH-staining (=somatostatin/neuropeptide Y) neurons, and a sparing of cholinergic neurons, especially in the centre of the injection site (Davies and Roberts, 1988). This is supported by Boegman and co-workers (1987) who injected quinolinic acid into the rat striatum and found that the NPY cells were eliminated and that the

cholinergic cells were resistant.

Mechanism of motor disorder

Initially the symptoms of Huntington's chorea were interpreted purely as a release phenomenon (Perry *et al.*1973; Hornykiewicz, 1979b), due to loss of the inhibitory neurotransmitter GABA, and the reduction of its effect upon the output structures of the basal ganglia. Current models are somewhat more complicated than this.

Taking the loss or dysfunction of GABAergic cells in the striatum (Graveland *et al.*1985a) projecting to the LGP (Reiner *et al.*1988) as the principal lesion, there would be an increase in the inhibitory influence of the LGP over the STN. This would produce a reduction of excitatory effect upon the MGP from the STN, and a loss of MGP inhibition of the thalamocortical pathway. The consequent overactivity of thalamocortical pathway would increase activation of the cortex, resulting in excessive disordered movements.

Injection of a GABAergic antagonist into the medial putamen (Crossman *et al.*1988) produces chorea (see below), which suggests that there might be a common mechanism with more specific choreas.

Other causes of dyskinesias and chorea

Wilson's disease - hepatolenticular degeneration (Wilson, 1912) - is a disorder of copper metabolism which can cause symptoms of motor system overactivity such as chorea. There is impaired biliary excretion of copper, and it accumulates in the liver, brain, kidneys, and cornea where it deposits to form the diagnostic brown Kayser-Fleischer ring. In the brain the deposits are in the basal ganglia, which atrophy and cavitate. Symptoms reflect a variety of basal ganglia dysfunctions, from parkinsonism to hyperkinesias. There may also be psychological symptoms, with personality changes, dementia and schizophreniform symptoms.

Chorea is a part of several other disorders with specific neurological consequences, ataxia-telangiectasia, Lesch-Nyhan syndrome (a disorder of purine synthesis), chorea-acanthocytosis, and benign hereditary chorea.

Infection with streptococcus group A can cause an encephalitis associated with a chorea which lasts 2-6 months, Sydenham's chorea (also known as St. Vitus' dance), which can be controlled by antidopaminergic agents.

As mentioned above L-dopa and the dopamine-antagonistic antipsychotic agents can cause dyskinesia/chorea.

Phenytoin and oestrogen interact with dopamine receptors and can have similar effects. Thyrotoxicosis can produce dyskinesia, and it is seen in pregnancy rarely (chorea gravidarum). Systemic lupus erythematosus and polycythaemia rubra vera, with widespread blood vessel lesions, can produce a variety of dyskinesias, as can cerebrovascular disease.

If a drug has been identified as the cause, this should be withdrawn or decreased if possible. Otherwise, antidopaminergic agents are the first line of treatment in these disorders, although success is variable. There has been little success with attempts to use GABA agonists in the treatment of dyskinesias, probably because a large number of cells in the caudate nucleus/putamen and in both parts of the globus pallidus are GABAergic and thus the effect is very nonspecific.

Interpreting HD as the neurochemical/pathological opposite of PD leads to the therapeutic use of dopaminergic antagonists in HD. The model was that dopamine acts to inhibit the inhibitory GABAergic output path, and so dopamine antagonists would block the action of dopamine upon these cells. However, as the striatal cells upon which dopamine acts are lost, this therapy has had limited success.

Hemiballismus - Subthalamic nucleus

Hemiballismus has gained particular attention among basal ganglia dyskinesias, out of proportion to its frequency of occurrence, as it is produced by a lesion of one discrete area. This disorder reflects the removal of one specific group of cells - the subthalamic nucleus and its projections, to the medial and lateral GP and SNr (Carpenter *et al.*1981). A lesion of the subthalamic nucleus causes uncontrolled violent flailing movements, usually involving the proximal upper limb and axial muscles of the contralateral side. Clinically this is often due to ischaemic cell death following a cerebrovascular accident - haemorrhage or infarction of the contralateral subthalamic nucleus. It is not a common disorder, but does correlate with hypertension and atherosclerotic disease. Usually it is self-limiting, and can be controlled with anti-dopaminergic anti-psychotic agents.

Animal models

Experimental lesions which destroy the subthalamic nucleus or its projections to the MGP or the SNr can cause hemiballismus in primates (e.g. Hammond *et al.*1979). Occasionally it is produced by lesions related to the STN by a polysynaptic pathway.

Crossman and co-workers (Crossman *et al.*1980; 1984;

1988) produced dyskinesia in primates by injections of GABA antagonists, picrotoxin and bicuculline into the subthalamic nucleus and the LGP. This effect in the STN is not readily explicable in terms of a simple sequential chain of inhibition/excitation, with current knowledge of the neurotransmitters involved. One explanation is that the inactivation of the STN is due to a depolarisation block. The effect of injections into the LGP would be disinhibition of GABAergic pallidosubthalamic cells. This would inhibit the cells of the STN, which would reduce stimulation of the MGP.

A similar dyskinesia was produced from injections of GABA antagonist into the medial putamen (Crossman *et al.*1988). The mechanism of this dyskinesia might be similar to that of Huntington's disease, with a loss of striatal GABAergic function. The GABAergic projection neurons are known to have local axon collaterals which arborise in striatum (Marco *et al.*1973; Park *et al.*1980; Wilson and Groves, 1980; Lighthall *et al.*1981; Kawaguchi *et al.*1989), and there are also GABAergic interneurons, and the local inhibitory effects of these cells would be lost. Lateral inhibition could be an important mechanism for reducing inappropriate neuronal activity, and thus GABA antagonists would block this.

This model is supported by Robertson *et al.*(1989) who describe hemiballismus following the injection of antagonists

of glutamate into the medial GP in primates. This would block the excitatory input of the STN, with decreased inhibitory MGP-thalamic activity.

Mechanism of motor disorder

Mitchell and co-workers (1985) used 2-deoxyglucose experiments to study the activity of pathways in the primate model of dyskinesia generated by GABA-antagonist injection into the STN. They observed a decrease in activity in the STN, MGP and LGP, the SN, the ventromedial and ventrolateral thalamic nuclei. Similar results were obtained following injection of GABAergic agonists and antagonists into the rat STN (Feger and Robledo, 1991).

There has been conflicting evidence regarding the nature of the subthalamic neurotransmitter, and thus the nature of the lesion in hemiballismus. The theory was that the STN-MGP pathway was inhibitory, thus lesioning would produce a release phenomenon. However, it now seems that the neurotransmitter involved, to both parts of the GP, is glutamate (Kitai and Kita, 1987; Smith and Parent, 1988; Parent *et al.*1989).

Removal of excitation of MGP-thalamic cells results in a decreased activity; these cells are inhibitory upon the VA/VL thalamus, so there is increased activation of the thalamocortical path, which results in hemiballismus.

Tics

Tics and Gilles de la Tourette's Syndrome (De la Tourette, 1885 cited in Shapiro *et al.*1978), are classified as hyperkinetic disorders.

Tics are sudden stereotyped movements, usually of the face and neck, and Tourette's Syndrome includes also repetitive vocalisations "phonic tics" - often of obscene words or phrases (coprolalia). The onset is characteristically between 2-15 years of age, of unknown aetiology, although it may be associated with encephalitis or neuroleptic withdrawal (Klawans *et al.*1978). There are no anatomical abnormalities found *post mortem*, and there may be a strong psychological component. Frequency is increased by stress and fatigue, they are absent during sleep, and are characteristically suppressible by voluntary effort. These are far more complex, coordinated movements than choreas or tremors. The complexity of the actions suggests that the lesion in these cases is at a higher level than with other motor disorders.

As with the previously-described disorders of movement the balance between dopaminergic and cholinergic activity has been a focus for therapeutic intervention. In this group of disorders, blockers of dopaminergic action seem to have been the most succesful, although again success has been limited and variable.

The drugs most often used are antidopaminergic agents such as haloperidol or pimozide (Shapiro *et al.*1978). In some cases a specific D2 antagonist seems to be especially effective (Uhr *et al.*1984). As would be expected, motor side-effects, such as akithisia and drug-induced parkinsonism can limit this form of therapy. Amphetamine-induced stereotypy seen in humans, treatable by neuroleptics (Young *et al.*1982) seems to be caused by a very similar mechanism. Potentiation of cholinergic transmission, using the cholinesterase inhibitor, physostigmine, has sometimes been effective (Shapiro *et al.*1978).

Animal models

Stereotyped behaviours can be produced in animals by administration of dopaminergic agonists. Attempts have been made to dissect out different components attributable to actions at different dopamine receptors, such as rat grooming being produced by D1 agonists, and sniffing by D2 agonists. Difficulties have arisen from finding increasingly that drugs are less specific than was previously thought, and that they have different effects in different species.

Dystonia

Dystonia is characterised by muscular activity which is

more tonic in nature than some of the preceeding disorders. Movements are twisting with rapid spasms of contraction. Joints and posture become progressively distorted. Facial and neck muscles may be involved, and there may be blepharospasm. Some relief may be obtained with anticholinergic agents. Dystonia can occur as a hereditary illness or as part of the spectrum of symptoms in disorders such as Wilson's disease, HD, encephalitis, manganese or cobalt poisoning, after head injury, or following L-dopa or phenothiazine therapy.

Athetosis

Athetosis is characterised by involuntary writhing movements - mobile spasms, often of the face or upper limb. The movements are less violent but more hypertonic than in chorea, and may be better classified as dystonia than chorea. During voluntary activities there appear to be "overflow" movements, as if fragments of inappropriate motor patterns intrude into the sequence by some mechanism of disinhibition. Speech can be impaired in this way. There is no effective treatment. Clinically athetosis is seen following anoxic brain damage, for example after perinatal trauma causing cerebral palsy, or due to cerebrovascular accidents. There is no clear indication of a specific site of lesions in these cases; although they often involve nuclei of the basal ganglia, or their output pathways, this is not always the case.

Species differences

Proportionately far more of the sensory-motor cortex is devoted to the face and hands in primates than in lower species. Double-labelling studies provide evidence that more axon collateralisation is seen in lower species. This would mean less flexibility of control over motor commands in these species. In primates, much finer control of muscle groups and more sophisticated movements are possible, and disorders of movement reflect this. The defect in the unilateral 6-hydroxydopamine-lesioned rat would seem to be generated by a simpler mechanism than that which produces the disordered control of movement in the parkinsonian human. The MPTP-lesioned primate is closer, but extensive studies have yet to reveal the precise functional nature of the lesion.

It has been difficult to find precise animal models for most human disorders of movement. This is undoubtedly a reflection of a more developed control of motor function in higher primates combined with the paucity of knowledge regarding pathogenesis. Partial models have been developed in a number of cases, but interpretations of studies utilising these models must take into consideration their limitations.

Summary

Disorders of movement have been interpreted as a continuum, from akinesia and bradykinesia, through normal function, to dyskinesia and hyperkinesia with increasing disruption of motor function at either end of the spectrum. Studies have attempted to understand the roles of different nuclei of the basal ganglia by examining the effects of lesions and of injections of neurotransmitter agonists and antagonists at different sites. The interpretation of these experiments, and of *post mortem* examination of human tissue has led to a basic model of the patterns of pathway excitation and inhibition in human disorders of movement. Despite the current level of understanding, the extrapolation of this model to the treatment of humans has met with many obstacles.

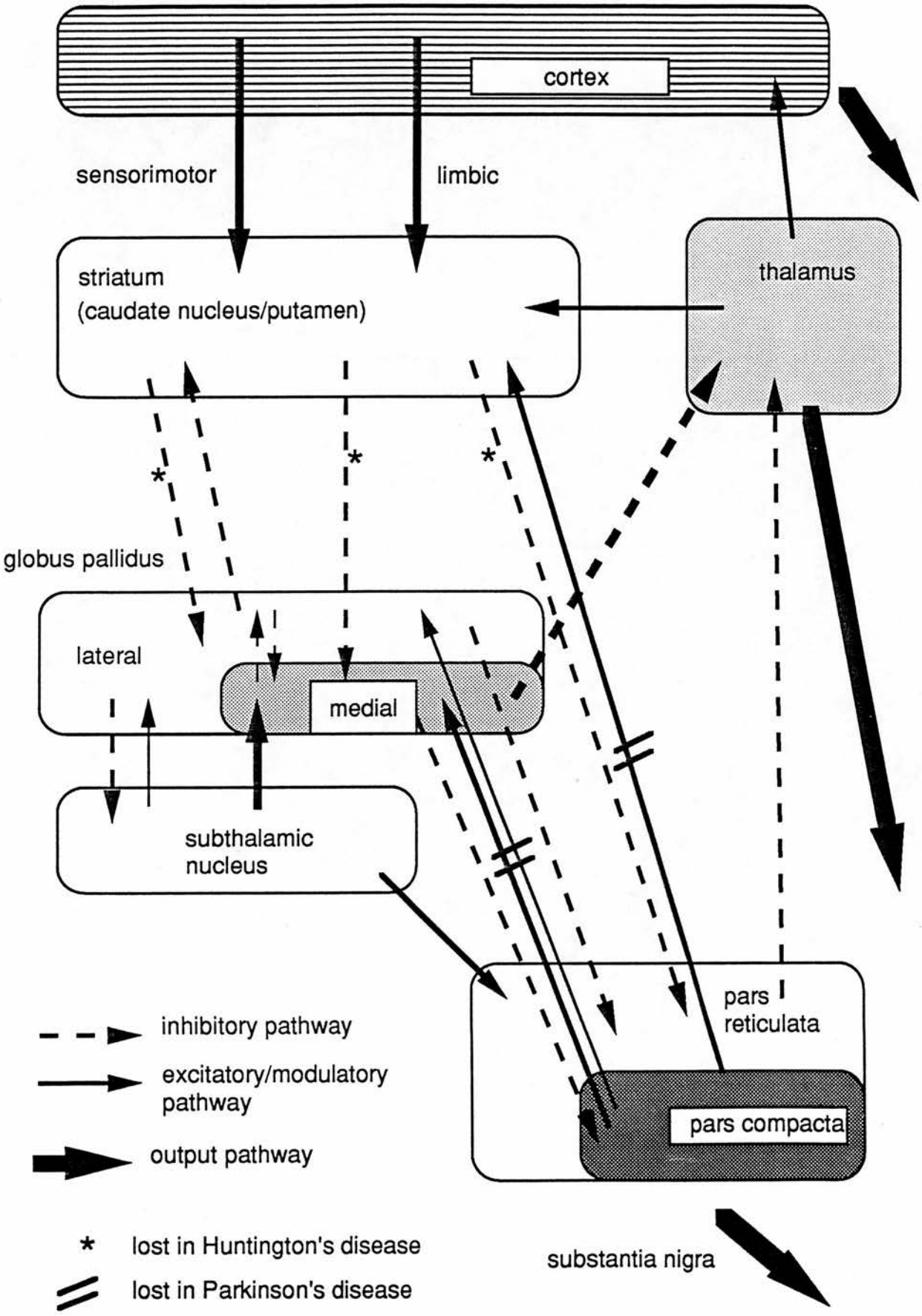
Aims and objectives of this thesis

The first part of this thesis is concerned with the anatomical and electrophysiological demonstration of the pallidostriatal pathway in the rat. There a number of loops between pairs of nuclei within the basal ganglia, and it is likely that feedback control plays a major part in the regulation of neuronal activity. The pallidostriatal connection is reciprocal to the large striato-LGP projection, which degenerates early in Huntington's disease, and its disinhibition could play an important role in the mechanism of movement disorder.

In the second part of the thesis I developed a method of filling cells with dye, with the initial aim of studying the cholinergic interneurons of the striatum. Cholinergic functions are implicated in various disease processes, although the role of this cell type is not yet clear. The method did not, however, prove successful for studying these cells, but did permit the study of the dendrites of the medium-sized spiny projection neurons of the striatum, which make up a very high percentage of the striatal neuronal population. Different studies have produced differing descriptions of the relationship of the dendritic arbours of these cells to the histochemical subdivisions of the striatum, striosomes. These compartments also reflect an organisational subdivision in that they seem to play different functional roles and are differentially affected in disease states. A description of the relationships betweeen medium spiny cells and striosomes would provide important information about the anatomical organisation of the striatum, with consequences for the understanding of striatal function.

An unexpected finding during the analysis of the data was that the dendritic fields of medium spiny cells were strikingly oriented. This appears to correlate with the orientation of other striatal structures, and also has functional implications.

Figure 1.1 Summary of basal ganglia connections



Chapter 2

The globus pallidus

Anatomy

Introduction

The globus pallidus is situated medially, ventrally and caudally adjacent to the striatum and is intimately related to it, both anatomically and functionally. The large numbers of myelinated fibres passing through it give it its pale appearance and name. The globus pallidus is separated from the putamen by the fibres of the internal capsule here called the external medullary lamina. (These two structures, GP and putamen, together are called the lentiform nucleus.) In higher mammals, such as primates, the globus pallidus is subdivided by the internal medullary lamina, formed by fibres of the internal capsule, into the lateral or external and the medial or internal segments. In subprimates (e.g. rats, cats and ferrets) the internal segment is represented by the entopeduncular nucleus, which is a small ovoid structure embedded in the descending fibres of the internal capsule forming the cerebral peduncles. In these species the term "globus pallidus" usually refers to a structure homologous with the primate lateral globus pallidus.

Cell types

The cell types are basically the same in external pallidum/GP and internal pallidum/EP.

Large cells

The majority of cells in the pallidal structures of all species are relatively large neurons; these are the source of most efferents of the globus pallidus. In the mouse (Iwahori and Mizuno, 1981a) these cells are 25-28um in diameter and stellate or multipolar, with five to seven primary dendrites. In the rat they have been described with the smaller dimensions of 12 x 20um to 15 x 30um (Park *et al.*1982) with similar numbers of primary dendrites. These make up about 70% of the cell population (rat; van der Kooy and Kolb, 1985). Park and co-workers (1982) describe differing dendritic and axonal distributions of laterally and medially located large neurons. Lateral cells form disc-like dendritic trees parallel to the external medullary lamina, without local axon collaterals, and medial cells form cylindrical dendritic trees oriented approximately radially with local axon collaterals. In the cat (Iwahori and Mizuno, 1981b; Dvergsten *et al.*1986) the typical large cell is conical, with an average diameter of between 24 x 59um, with 3-6 primary dendrites. The latter group of authors, working with three-dimensional reconstructions found no specific orientation to the dendritic trees of neurons of the cat EP, which were often spherical or discoid.

In the primate (Fox *et al.*1974a cited in DiFiglia *et al.*1982, Francois *et al.*1984; Yelnik *et al.*1984) the cell body dimensions are 20-50um in diameter, with long

axons and long, sparsely-branched dendrites with many spines and appendages. No differences were found between medially and laterally located cells.

A second type of large cell described only by DiFiglia *et al.* (1982) in the primate has a globular body, with radially oriented dendrites.

The vast majority of primate pallidal cells are positive when stained by antibodies to GABA (Smith *et al.*1987), and GABA has been identified as the neurotransmitter for most, if not all, of the pallidofugal pathways (see below). Some of these cells also contain substance P (rat, Kanazawa *et al.*1977).

Medium cells

In the mouse, Iwahori and Mizuno (1981a) describe another cell type which occurs frequently - medium-sized neurons, spindle or fusiform in shape, with diameters of 19-27um and three to five primary dendrites. They found the cellular composition of the entopeduncular nucleus to be similar to that of the globus pallidus. In the cat (Iwahori and Mizuno, 1981b) the medium size cells, which tend to be located ventromedially, are piriform with dimensions of 18 x 29um, and 3-6 primary dendrites.

In the macaque and human, Percheron and co-workers (Francois *et al.*1984; Yelnik *et al.*1984), describe a

medium-size (23-24um in diameter) neuron with a triangular soma and a long axon which they consider to be a projection neuron. These cells were located only in the rostral pole and lateral border of the MGP and may correspond to the pallidohabenular neurons described by Parent and De Bellefeuille (1982).

Small cells

Van der Kooy and Kolb (1985) described a small cell in the rat GP (12-20um), which they found to project to the cortex, distinct from the surrounding cortically-projecting cholinergic cells of the nucleus basalis of Meynert (Divac, 1975; Kievit and Kuypers, 1975; Lehmann *et al.*1980; Parent *et al.*1981; Rye *et al.*1984).

DiFiglia and co-workers (1982) describe in the primate a less numerous group of smaller cells (12-21um) with locally arborising axons which they believe to be inter-neurons; they found far fewer of these in the medial segment of GP than in the lateral segment.

These authors (DiFiglia *et al.*1982) suggest that the different cell types seen in anatomical studies correspond to cells with different electrophysiological characteristics seen by Delong (1971) and Filion (1979). In the lateral pallidal segment two types of spontaneous discharge patterns were observed; 85% of units showed bursts of high frequency discharge separated by intervals of several seconds of silence. It seems likely

that these would correspond to the large cell type. 15% of cells fired with a lower frequency of discharges and bursts; these might be interneurons modulating the activity of the large cells. In the medial pallidal segment all cells tended to fire continuously, although not always with a constant rate; this would correspond to a population comprising mainly a single cell type, i.e. large, without interneuronal modulation.

Afferents to GP/LGP

Striatum

The globus pallidus/LGP receives a major topographically-related input from the striatum (Voneida, 1960; Szabo, 1967, 1970; Nauta and Mehler, 1966; Royce and Laine, 1984; Yelnik *et al.*1984), from the medium-sized densely spiny cells (Kemp and Powell, 1971a; Fox and Rafols, 1975; Grofova, 1975; Preston *et al.*1980; Chang *et al.*1981). This projection appears to arise from a separate cell population from the similarly massive striatal input to the MGP (Beckstead and Cruz, 1986; Parent *et al.*1989b). The axons enter perpendicularly to the external surface of the GP. In the rat (Wilson and Phelan, 1982) the axons of these cells form two dense arborisations, one just within the border of the GP, branching out perpendicularly to the main course of the axon, and the second deeper within the GP. These have been seen as bands of anterograde

labelling in the primate (Smith and Parent, 1986a; Hedreen and Delong, 1991). These arborisations correspond morphologically with the dendritic fields of the large pallidal cells (Percheron *et al.*1984). The authors of this research, in the macaque, suggest that the pallidum acts as a kind of funnel for neuronal information from the striatum, with each pallidal cell integrating the signals from a number of striatal cells.

In the rat 40% of these cells have collaterals to the SN/VTA (Loopuijt and van der Kooy, 1985).

Many of the striatal projection neurons contain GABA and it has been demonstrated (Fonnum *et al.*1978a; Nagy *et al.*1978a; Pan *et al.*1983, 1985) in the rat using lesion studies that this pathway seems to be mediated by GABA. Ribak *et al.* (1979) used immunocytochemical methods which also indicated this.

Obata and Yoshida (1973) demonstrated in the cat pallidum *in vivo* that iontophoresis of GABA caused an inhibition of firing. Similarly, other results from *in vivo* experiments involving electrical stimulation of the striatum, indicate that it has an inhibitory influence upon the pallidum. Extracellular recordings from the primate pallidum (Ohye *et al.*1976) showed varying patterns of inhibition and excitation following striatal stimulation. Intracellular recordings in cat (Malliani and Purpura, 1967; Yoshida *et al.*1972; Levine *et al.*1974)

and rat (Park *et al.*1982; Nakanishi *et al.*1985 - in brain slice preparation) showed a hyperpolarisation following striatal stimulation.

There are also high levels of immunoreactivity for met-enkephalin in the LGP/GP (Haber and Elde, 1981; Haber and Elde, 1982; Haber and Nauta, 1983; Haber and Watson, 1985) in projections from the striatum (Cuello and Paxinos, 1978; Haber and Elde, 1982; Del Fiacco *et al.*1982). From electrophysiological recordings in the rat GP (Napier *et al.*1983a; Napier *et al.*1983b) the inhibition of spontaneous activity caused by striatal stimulation was blocked by the opiate antagonist naloxone. Administration of morphine caused a decrease in spontaneous firing rate.

After making excitotoxin lesions in the cat striatum (Sachdev *et al.*1989) there is an increase in the mean firing rate of pallidal (i.e. LGP) cells and a concomitant decrease in the variability of firing rate, i.e. less tendency to burst. This suggests that there is a tonic inhibitory striatopallidal projection which also performs some sort of regulatory function.

Bursting in normally innervated cells may be the result of activation of the low-threshold calcium-dependent current described by Jahnsen and Llinas (1984) in thalamic cells *in vitro*. This current is activated at a

small hyperpolarisation from resting membrane potential in these cells and results in depolarising waves which can give rise to bursts of action potentials.

Subthalamic nucleus

A large input to the LGP from the subthalamic nucleus has been descibed in most species (Ricardo, 1980; Carpenter *et al.*1981a; Carpenter *et al.*1981b; Kita *et al.*1983a; Kita and Kitai, 1987; Parent *et al.*1989). Smith and co-workers (1990) demonstrated bands of labelling in GP parallel to the medullary laminae, as seen with the striatopallidal projection. At higher powers these terminals surrounded pallidal cell bodies.

Many subthalamic efferents have collaterals, although there are variations in their patterns of branching between species. In the rat Deniau *et al.* (1978) demonstrated using electrophysiological techniques that many cells projecting to GP (and/or to EP) had collaterals to the substantia nigra. Van der Kooy and Hattori (1980), using retrograde double-labelling techniques in the rat, supported these results, and found that the double-labelled cells accounted for almost all of the cells in this nucleus.

In the cat (Beckstead, 1983) and in the primate (Smith and Parent, 1986a), a subthalamostriatal projection has been described, although other studies have not found this to be significant (Carpenter and Jayaraman, 1991).

This originates from the same part (dorsolateral) of this nucleus as the projection to the GP. This raises the possibility of another system of collaterals (Smith and Parent, 1986a). In the rat, this projection is described as "moderate" (Kita and Kitai, 1987), or is not seen (Jackson and Crossman, 1981).

Carpenter and Jayaraman (1991) demonstrated in cats and squirrel monkeys that most cells projecting to LGP also project to MGP, although this contradicts evidence from Parent and co-workers (1989b) that very few cells project to both nuclei in the primate.

Previously most evidence has suggested that the neurotransmitter of subthalamic cells projecting to LGP is GABA (e.g. Perkins and Stone, 1981, 1980; Crossman *et al.*1980, 1984; Mitchell *et al.*1985). More recent electrophysiological and immunohistochemical studies (Kita and Kitai, 1987; Parent *et al.*1989a; Feger *et al.*1989) have indicated otherwise. Smith and Parent (1988) demonstrated glutamate but not GABA immunoreactivity in the STN, except in afferent nerve terminals (Smith *et al.*1987). Hamada and Delong (1988) showed a decrease in LGP cell activity following lesions of the STN in primates, which would correlate with this pathway being excitatory.

Thalamus

Kincaid and co-workers (1991) have demonstrated, using anterograde and retrograde tracing, an input from the parafascicular nucleus of the thalamus. This site receives a projection from MGP, and thus this might be another route (with the subthalamic nucleus, and direct connections) by which the two components of the pallidal complex might influence one another.

Midbrain

There is a large dopaminergic input from the SNc to MGP, which has a smaller component to LGP (Parent and Smith, 1987). These are distinct from the nigrostriatal pathway, unlike in the rat (Lindvall and Bjorklund, 1979) where this input is composed of branches of the nigrostriatal axons. A serotoninergic input from the dorsal raphe nuclei has been demonstrated (Parent *et al.*1981; Pasik *et al.*1984; Parent *et al.*1991).

There is a projection from the pedunculopontine nucleus to both the contra- and ipsi-lateral GP in rat (Jackson and Crossman, 1983). This was shown to be excitatory in the cat by Gonya-Magee and Anderson (1983). The identity of this projection site has been disputed by Rye and co-workers (1987), who identify it by immunohistochemistry to cholineacetyltransferase (ChAT) in the rat. They distinguish the ChAT-positive cells from the cells receiving input from the GP, EP and SNr, and call the latter the midbrain extrapyramidal area.

MGP/EP

A small reciprocal connection between the two pallidal segments was described in the primate by Hazrati and Parent (1990).

Efferents of GP/LGP

Subthalamic nucleus

The external segment/GP is known to project predominantly to the subthalamic nucleus in all species studied (rat, cat, and primate)(Wilson, 1914; Mettler, 1944; Nauta and Mehler, 1966; Carpenter *et al.*1968; Hattori *et al.*1973; Kim *et al.*1976; Carter and Fibiger, 1978; Nauta, 1979; McBride and Larsen, 1980; Perkins and Stone, 1980, 1981; Carpenter *et al.*1981a, 1981b). Van der Kooy and Kolb (1985) estimate that 70% of pallidal cells in the rat project to this site.

This projection is topographically organised (Carpenter *et al.*1968; McBride and Larsen, 1980; Carpenter *et al.*1981a, 1981b), and makes up the efferent half of the pallidosubthalamopallidal loop. This projection is apparently inhibitory, demonstrated by extracellular recording in primates (Ohye *et al.*1976) and in rats (Perkins and Stone, 1981; Kita *et al.*1983b). There is strong evidence that it is GABAergic (Fonnum *et al.*1978b; Perkins and Stone, 1981; Vincent *et al.*1982), although Takada and Hattori (1987) demonstrated

transport of radio-labelled glycine and not GABA along this pathway.

Substantia nigra

The LGP projects also to the substantia nigra (Nauta and Mehler, 1966; Grofova, 1975; Hattori *et al.*1975; Kanazawa *et al.*1976; Nauta, 1979; McBride and Larsen, 1980; Parent and De Bellefeuille, 1983; Totterdell *et al.*1984; Smith and Bolam, 1989) predominantly to the pars reticulata but also to the dopaminergic neurons of the ventral tegmental area and the pars compacta.

This projection has been shown, in the rat, to be GABAergic by indirect lesion studies and by immunohistochemistry (McGeer *et al.*1971b; Hattori *et al.*1973; Brownstein *et al.*1977; Nagy *et al.*1978a; Ribak *et al.*1980; Araki *et al.*1985; Smith and Bolam, 1989). Smith and Bolam (1990a) recently demonstrated GABAergic terminals from the rat GP, identified by anterograde tracing, making close contact with cell bodies of the SNr projection neurons, and also with dopaminergic cell bodies.

There is a reciprocal connection with the pedunculopontine tegmental nucleus (Hammond *et al.*1983), the identity of which is disputed, as described above (Rye *et al.*1987).

Striatum

The possibility of a projection to the striatum was noted in anatomical studies in the primate by Wilson in 1911, and later by Mettler in 1943. Nauta's autoradiographic studies in the cat (1979) documented this projection and Staines and co-workers (1981) demonstrated pallidostriatal connections using retrograde transport of horseradish peroxidase conjugated to wheat germ agglutinin. The projection was only visible after blockade of anterograde labelling with kainic acid since otherwise the dense terminal network obscured the cell bodies. This result suggests a precise topographic relationship between the projections. This projection has been further characterised as making contact with the somatostatin interneurons of the striatum (Staines and Hincke, 1991). Jayaraman in 1983, using retrograde transport of horseradish peroxidase, also described a topographical relationship between the rat GP and striatum. The description of pallidostriatal cells with axon collaterals to the SN in the rat (Staines and Fibiger, 1984) led to the suggestion that these cells might coordinate striatal and nigral activity.

Takada and co-workers (1986) described pallidostriatal cells in the rat with collaterals to the thalamic paraventricular nucleus. Cells projecting to this nucleus were also seen by van der Kooy and Kolb (1985). They retrogradely labelled a population (14% of GP

neurons) of GP cells from frontal and prefrontal cortex injections and approximately half of these sent axon collaterals to the thalamic paraventricular nucleus. These cells did not have collaterals to the subthalamic nucleus and tended to be smaller (12-20um) than the other GP cells.

Thalamus

There is a small but significant LGP projection to the paraventricular nucleus of the thalamus, (Sugimoto and Hattori, 1984; van der Kooy and Kolb, 1985; Takada *et al.*1986). This projection seems to be made up of axon collaterals (see above). The thalamic paraventricular nucleus projects to the nucleus accumbens, and thus these pallidal cells might play a role in the integration of sensorimotor and limbic systems.

Cortex

Van der Kooy and Kolb (1985) identified, using fluorescent retrograde tracing in the rat, a population of small pallidal cells which were labelled from cortical injections (see above). The large cholinergic cortically projecting cells of the nucleus basalis of Meynert may lie within the outer borders of the globus pallidus in the rat (Divac, 1975) and the cat (Parent *et al.*1981), and thus deserve mention in this section.

MGP/EP

Hazrati and Parent (1990) have described reciprocal connections between the two pallidal segments, using anatomical tracers in the primate. At the electron microscope level, Smith and Bolam (1990b) have demonstrated terminals from rat GP cells making contact with cells in the EP.

Afferents of MGP/EP

Striatum

Like the LGP/GP, the EP/MGP receives its major input from the striatum (Voneida, 1960; Szabo, 1967; Szabo, 1970; Kemp and Powell, 1971a; Fonnum *et al.*1978a; DeVito *et al.*1980; Royce and Laine, 1984; Moriizumi *et al.*1987) from a different group of cells than those which project to the LGP (Beckstead and Cruz, 1986; Parent *et al.*1989b).

This projection is inhibitory and GABAergic (Obata and Yoshida, 1973; Nagy *et al.*1978a; Pan *et al.*1983, 1985) from medium-sized densely spiny cells, a number of which also appear from immunohistochemical studies to contain substance P (Kanazawa *et al.*1977; Haber and Elde, 1981; Bolam *et al.*1983; Beckstead and Kersey, 1985; Haber and Watson, 1985) and/or dynorphin (Graybiel and Chesselet, 1984).

In electrophysiological studies upon cells of the

substantia nigra, substance P seems to have an excitatory effect (Davies and Dray, 1976; Pinnock *et al.*1983), thus the combined effect of GABA and peptide neurotransmitter upon the pallidal target is not immediately obvious. Like the projection to LGP, this striatal influence is probably regulatory with a generally inhibitory action. Stimulation of the striatum inhibits the spontaneous activity recorded extracellularly in the cat EP (Obata and Yoshida, 1973). In the conscious primate Ohye and co-workers (1976) recorded a variable response often consisting of an inhibition followed by a rebound excitation.

After excitotoxin lesions of cat striatum (Sachdev *et al.*1989) the firing rate was not changed, although as in the LGP, the variability of rate was decreased. As GABA is the common neurotransmitter of the striatal efferents to LGP and MGP, and the common effect in both these nuclei is a decrease in excitability, this evidence would suggest that GABA is responsible for the bursting activity of LGP and MGP cells (via the low-threshold calcium current). The loss of the substance P input does not have a gross effect upon the cells in this experiment. Maybe the rate of firing is controlled by the other major input, i.e. the glutamatergic input from the subthalamic nucleus. In the LGP there is an increase in mean firing rate following striatal lesions (Sachdev *et al.*1989), which suggests that the

enkephalinergic input is responsible for maintaining the rate in this segment.

LGP/GP

Smith and Bolam (1990b) demonstrated GABAergic terminals from the rat GP converging with terminals from the striatum upon cell bodies in EP.

Midbrain

In the squirrel monkey an input from the dopaminergic midbrain cells, the SN/VTA group has been described (DeVito *et al.*1980; Smith *et al.*1989). In the primate this input is distinct from the dopaminergic input to LGP (Parent and Smith, 1987), and there was little evidence that this projection was due to collaterals of nigrostriatal cells, unlike in the rat (Lindvall and Bjorklund, 1979).

Jackson and Crossman (1983) used both anterograde and retrograde tracing to label afferents to rat entopeduncular nucleus from the ipsilateral pedunculopontine nucleus. In the cat this was shown electrophysiologically to be excitatory (Gonya-Magee and Anderson, 1983). This was demonstrated anatomically in the primate by DeVito and co-workers (1980), who also observed an input from the dorsal raphe nucleus (also Parent *et al.*1991).

Subthalamic nucleus

The subthalamic nucleus projects to both pallidal segments (Nauta and Cole, 1978; Ricardo, 1980; DeVito *et al.*1980; Carpenter *et al.*1981a, 1981b; Kita and Kitai, 1987; Parent *et al.*1989a; Smith *et al.*1990). The branches to the EP in the rat are often collaterals of axons to other nuclei such as the SN and the GP (Deniau *et al.*1978; Hammond *et al.*1983). A more recent retrograde fluorescent double-labelling study in the primate (Parent *et al.*1989b) suggests that in higher species the two pathways are segregated.

The pharmacological evidence as to the neurotransmitter involved has been conflicting, as the clinical picture of hemiballismus would seem to indicate a removal of an inhibition. Electrophysiological experiments (Rouzaire-Dubois *et al.*1983; Crossman, 1987) indicated that this pathway was GABAergic and thus inhibitory, and blocked by antagonists of GABA. However, it is not clear that these results were uncontaminated by simultaneous stimulation of other pallidal afferents, which are known to be GABAergic. More recent electrophysiological experiments indicate that it is excitatory (Kitai and Kita, 1987; Hamada and Delong, 1988). Immunohistochemical evidence shows that the subthalamic nucleus-MGP pathway is glutamatergic (Smith and Parent, 1988; Parent *et al.*1989a).

Efferents of MGP/EP

Thalamus

The major targets of the internal segment/entopeduncular nucleus, are the thalamus - ventroanterior-ventrolateral complex (VA/VL), centre median-parafascicular complex (CM-pf), the lateral habenular nucleus and the midbrain - pedunculopontine nucleus pars compacta. The size, distribution, and degree of collateralisation varies between species.

In the rat (van der Kooy and Carter, 1981) the cells of caudal third of the nucleus project to the VA/VL thalamus, the CM-pf complex and the tegmental pedunculopontine nucleus. These projections were demonstrated in the primate by DeVito and Anderson (1982), who also found the MGP-thalamic connection to be topographically organised. Most of these cells send collaterals to at least two of these sites. This has also been demonstrated by collision testing in the cat and primate (Filion and Harnois, 1978; Harnois and Filion, 1980, 1982). These cells have been described anatomically in the primate by Parent and de Belle-feuille (1982, 1983). The cells of the central portion of MGP project to VA/VL and have collaterals to CM-pf.

In electrophysiological recordings in the anaesthetised cat, Uno and co-workers (1978) describe monosynaptic inhibition of VA/VL cells following EP stimulation.

This is corroborated by histochemical studies in the rat showing that the MGP-thalamic projection utilises GABA as its neurotransmitter (Penney and Young, 1981). This is one of the major points at which the basal ganglia have a direct influence upon motor control, via the inhibitory influence of EP upon the thalamocortical projection.

There is a relatively large topographical input to the lateral habenular nucleus (Nauta, 1974; Herkenham and Nauta, 1976; Filion and Harnois, 1978; Nagy *et al.*1978b). In the squirrel monkey (Parent and de Bellefeuille, 1982, 1983) the organisation of cells projecting to different areas is basically concentric, with the pallidohabenular projection arising from predominantly peripherally located cells. In the rat (van der Kooy and Carter, 1981) the entire rostral two thirds of the EP projects to the habenula, with no collateralisation. This projection utilises GABA (Nagy *et al.*1978b; Vincent *et al.*1982; Araki *et al.*1984). The habenula is considered part of the limbic system, and as such this is another point at which the basal ganglia can influence another system.

Midbrain

Collaterals from a number of MGP-thalamic cells project to the tegmental pedunculopontine nucleus pars compacta (Nauta and Mehler, 1966; Carter and Fibiger, 1978; Filion and Harnois, 1978; Parent and De Bellefeuille, 1982, 1983). There has also been described a projection to SNc from MGP/EP in cat (Grofova, 1975) and in primate (Nauta and Mehler, 1966; DeVito and Anderson, 1982).

There is no evidence that MGP projects to subthalamic nucleus in primate (Carpenter *et al.*1981a, 1981b).

Summary of LGP v MGP

The LGP seems to be distinct in function from the MGP in that it does not have a major projection out of the basal ganglia, but is more involved in the loops with the subthalamic nucleus, the striatum and the substantia nigra. In many aspects the LGP is similar to the SNc, and it seems to have more in common with this nucleus than the more closely anatomically related MGP. The activity of the LGP is mainly under the influence of the striatum, and is less affected by other inputs. Changes in the neuronal activity of the LGP can be interpreted as reflecting the activity of the internal loops within the basal ganglia, particularly those originating in the striatum.

The MGP has many similarities with the SNr in that it

projects out of the basal ganglia, one of the most important projections being to the thalamus VA/VL and CM-pf nuclei and lateral habenula. The activity of this nucleus can be interpreted as being an indicator of the output of the basal ganglia.

Many of the pathways in the basal ganglia are made up of axon collaterals. This would have the effect of coordinating the activity of the two or more target nuclei, and simplifying neuronal processes. In higher species, there is less collateralisation. This may be a mechanism whereby pathways are segregated from each other, permitting more sophisticated control of motor processes.

Investigation of pallidostriatal connectivity

Introduction

The LGP/GP striatal pathway has been described anatomically by several workers, as noted above, although usually as a peripheral observation. The existence of this path, reciprocal to the large striatopallidal connection, would provide a mechanism for feedback to the striatum from the globus pallidus. Similar reciprocal loops are seen between other structures of the basal ganglia, such as the striatum and the substantia nigra, and the subthalamic nucleus and the globus pallidus. It seems likely that these loops are an important mechanism for self-regulation of basal ganglia activity.

We studied this projection in the rat, using retrograde transport of fluorescent tracers. We also aimed to carry out an extracellular electrophysiological investigation of these neurons. In order to reduce the likelihood that we were observing pallidocortical neurons we performed a number of tests which would distinguish between these two populations of cells. Immunohistochemical staining for choline acetyltransferase (ChAT) identified, in the anatomical experiments, the cholinergic cortically-projecting cells which might have been labelled by fibre-of-passage uptake of the fluorescent tracer. In the electrophysiological experiments pallidostriatal cells were identified which had caudally projecting

collaterals to the crus cerebri, this was a means of distinguishing them from pallidocortical cells (van der Kooy and Kolb, 1985).

We looked at the electrophysiological characteristics of pallidal cells in an animal model of Parkinson's disease, the 6-hydroxydopamine (6-OHDA)-lesioned rat.

Methods

Neurophysiology

Sixty-four albino Wistar rats (200-250g) were anaesthetised with halothane, tracheostomised, and placed in a stereotaxic frame (Kopf) on an electrically heated blanket with a rectal probe to monitor body temperature.

The skull was exposed and burrholes made at the appropriate sites. A concentric stimulating electrode was positioned in the anterior striatum (coordinates calculated from Paxinos and Watson rat atlas; 1mm anterior and 2.5mm lateral to the bregma suture, and 5mm vertically below the surface of the dura). In 10 animals a second stimulating electrode was placed in the crus cerebri (at a location 4mm posterior, 2.5mm lateral to bregma, and 7.8mm vertically below the dura membrane) (figure 2.14(b)). Glass microelectrodes were filled with 2% Pontamine sky blue (Gurr) in 0.5M sodium acetate which gave resistances of 20-60MOhms measured at 1.5Hz.

Extracellular recordings were made from vertical electrode tracks aimed at the globus pallidus (0.8mm posterior and 2.5mm lateral to bregma). Individual neurons were identified either by spontaneous activity or by their response to electrical stimulation by one of the implanted electrodes. Interspike interval histograms were generated from a specified number of spontaneous spikes, eg 1000 for each cell. Post-stimulus histograms with a relatively long cycle (1+ seconds) were used to measure latency of response and its variation. These records were generated on-line with a computer (Arbuthnott *et al.*1984) and data were stored for further analysis.

Collision testing was performed to distinguish antidromic from orthodromic stimulation, in cells with both spontaneous and driven activity. A spontaneous spike was used to trigger the stimulus with varying latencies; if the response is being recorded from the axon of a striatal cell passing through the GP, a driven response will be seen at short latencies after the spontaneous spike. If, however, the stimulating electrode is located near the axon of a pallidal cell which passes through the striatum, the stimulus will generate an action potential travelling back towards the cell body. If at the same time a spontaneous spike is travelling in the opposite direction, from the cell body distally, the spikes will "collide" and the stimulated response will not reach the recording electrode, due to

inactivation of the sodium channels following the action potential. The interval between the spontaneous action potential and the stimulus which generates a spike at the recording electrode can be shortened until no response is seen at the recording electrode. Following frequency was tested to confirm that the unit could support stimulated activity at this frequency. This eliminated the false appearance of "collision" due to the inactivation period of the sodium channels.

In the experiments which employed two stimulating electrodes the various characteristics were described for stimulation from each electrode.

The end of each electrode track, at the ventral border of the GP was marked by passing a negative current (5uA for 5 minutes) through the recording electrode to deposit Pontamine sky blue in the tissue. From this mark it was possible to calculate the positions from which recordings had been made. The experiment was terminated after a maximum of three tracks; the animal was killed by a lethal dose of anaesthetic, and the brain was removed and frozen. Sections 40um thick were cut on a cryostat, mounted, fixed in formalin vapour overnight, and stained with various "Nissl" stains in order to determine the positions of the stimulating and recording electrodes. Only if the electrodes were in the correct sites were the results used.

Recordings were also made in seven animals which had received unilateral 6-hydroxydopamine injections (8ug in 4ul) into the medial forebrain bundle at least six months previously. When tested 5-9 days after the injection each animal turned more than 200 times contralaterally to the lesion during 45 minutes following administration of apomorphine (0.25mg/kg intraperitoneally). These animals can be expected to have depletion of more than 90% of dopamine of the lesioned side (Hefti *et al.*1980).

Neuroanatomy

Twelve male albino Wistar rats (200-250g) were used for the anatomical part of the investigation. Under Equithesin anaesthesia (16% nembutal (barbiturate), 4.2% chloral hydrate in a 4:1:3.4 polyethyleneglycol:alcohol:water mix; 3ml/kg i.p.) stereotaxic injections of 2% True Blue (Bentivoglio *et al.*1979) were made into the striata of eight rats. A total volume of 0.2ul was delivered through a 1ul Hamilton microsyringe, at coordinates 1mm anterior and 2.5mm lateral to bregma, and 5mm vertically below the dura membrane. In order to reduce spread up the needle track the injections were made over five minutes and the syringe was left in place for another two or three minutes before being slowly removed. Four rats received iontophoretic injections of 2% fluorogold into the striatum at the same coordinates using a 10-20um-tipped glass microelectrode.

After survival times of four to sixteen days, the rats were given lethal dose of anaesthetic and perfused through the heart with 0.1M sodium phosphate buffer (pH7.4) followed by 500ml 4% paraformaldehyde in the same buffer. The brains were removed, stored overnight in the same fixative, and then transferred to 20% sucrose for at least 24 hours for cryoprotection. Fifty micron thick sections were cut on a freezing microtome and collected in 0.5M Tris buffer (pH7.4). For some animals alternate sections were mounted on chrome alum/gelatin coated slides, air-dried, and examined under a fluorescence microscope. The parallel set of sections was immunostained for choline acetyltransferase (ChAT) by incubating overnight in a monoclonal antibody (a gift from Dr F. Eckenstein) 1:4000 in 100mM tris buffer (pH7.4) including 150mM sodium chloride, 2% bovine serum albumin, 10% rabbit serum, 0.5% Triton X-100, and 0.1% sodium azide. They were then rinsed in the same buffer and incubated for 30 minutes in 1:50 biotinylated rabbit anti-rat IgG (Vector). The rabbit secondary antibody was then visualised by reacting the sections with Avidin DH combined with biotinylated R-phycoerythrin (Vector). The last two incubations were repeated and then the sections were mounted on chrome alum/gelatin coated slides, dried in air and studied with a fluorescence microscope (Zeiss) with appropriate filter sets for fluorogold and phycoerythrin.

Results

Neurophysiology

Unlesioned animals

One hundred and thirty-nine cells were recorded from 25 rats, and were histologically confirmed to be in the globus pallidus. Of these 80 units were spontaneously active, of which 23 were driven antidromically from the striatum. 55 were silent, with the record being incomplete for the remaining 4 cells.

Spontaneously active cells

The distribution of firing rates among the spontaneously active neurons (for which the rates were recorded) is shown in figure 2.1. The firing rates of the spontaneously active GP neurons ranged from 0.6 to 76.6 impulses per second, with a mean of 16.0+/-14.8 spikes/second. In order to determine if the variation in firing rate was topographically dependent in any way, the positions of the fifteen slowest and the fifteen fastest firing cells were estimated from the marked electrode tracks and plotted on drawings from the atlas of the rat brain (Paxinos and Watson, 1986). There did not seem to be any specific location of cells at either extreme of the range of firing rate; figure 2.2 illustrates the positions of the 15 slowest and the 15 fastest neurons encountered.

The activity of the spontaneously active cells was affected in different ways by stimulation of the striatum. 26 cells responded with an orthodromic spike, and an increase of the spontaneous firing rate (5), or a decrease (3), or a mixed response, for example, an inhibition followed by a rebound excitation (11) (figure 2.3). (In the remaining 7 cells the records are incomplete and do not permit classification of the responses.) Thirty-one cells did not have a short-latency orthodromic spike but showed excitation (3), inhibition (9), a mixed response (5), or were not affected (14). Examples of different responses are shown in figure 2.4. The remaining 23 cells were antidromically driven and are discussed below.

There were no significant differences between firing rates of cells which had an orthodromic spike (mean rate +/-sd of 19.0+/13.1 spikes/second), an antidromic spike (12.2+/-12.5 spikes/second), or no driven spike (15.9+/-16.9 spikes/second); the distribution of the firing rates of these cells is shown in figure 2.5.

Orthodromic driving from striatum

Orthodromic activity with a recorded latency was seen in 26 spontaneously active cells, and in 51 otherwise silent cells (the latency was not recorded for 4 cells). Fourteen of the silent cells often responded to striatal stimulation with a burst of 2 to 5 regularly-spaced

spikes, usually decreasing slightly in size towards the end of the train (figure 2.6(a)). In only 2 of the spontaneously-active cells did stimulation evoke more than one spike (figure 2.6(b)). The numbers of spikes fired in response to stimulation was significantly different in the two groups p=0.046 by ANOVA.

The distribution of the latencies of the first driven spike is shown in figure 2.7. The extreme outlier, a silent cell with a latency of 180ms, has been excluded from subsequent calculations. For the spontaneously active cells the mean latency +/-standard deviation was 22.0+/-15.3ms (range 0.1-56ms); for the silent cells it was 10.9+/-8.9ms (range 3-50ms).

The mean latencies for the two groups were significantly different, using an ANOVA, with p=0.00015 (figure 2.8). The ratio of the variances of the latencies for the two groups, 235.1/79.9=2.94 was significant at a $p<0.01$ using Hartley's test (Myers, 1966, page 73). Thus the variance of the latencies of the spontaneously active cells was significantly greater than that of the silent cells. This suggests that these recordings were from two groups of cells which were under different influences from the striatum. One explanation is that the pathway from the striatum to the spontaneously active cells might be polysynaptic, which would account for the larger variation in latency of response.

Antidromic driving from striatum

Twenty-three pallidal cells were antidromically activated from striatum, as determined by collision of a stimulated spike with a spontaneous spike. This is shown in figures 2.9 and 2.10. The driven spike was characteristically of short latency (range 0.5-5ms, mean 2.4+/-1.4ms) (figure 2.11(b)) with little variation (less than 2ms) between stimulation cycles (figure 2.9(b)). The mean latency was significantly smaller than that of all other cells, $p<0.0001$ (figure 2.12(a)). Calculating the length of the path between pallidum and striatum this gives a conduction velocity of 0.3m/s. These cells were not distinguished from other pallidal units by their spontaneous activity rates (figure 2.5) or by their location in the GP.

None of the silent neurons excited from the striatum were included in this group as they could not be tested for collision. They sometimes appeared similar to antidromic cells as they followed at high frequencies of stimulation and occasionally had short relatively constant latencies (<2ms variability). However, as a population, they had different characteristics. The latencies of this group were significantly longer (mean 10.86+/-8.94ms, range 3-50ms) than for the collision-tested antidromically-driven group $p<0.0001$ (figure 2.12(b)). Comparing the variances using Hartley's test, the ratio was 79.92/1.88=42.51, which was highly

significant $p<<0.01$.

Crus stimulation

Thirty-one GP cells were recorded from 6 rats in which a stimulating electrode was placed in the crus cerebri, as well as in the striatum. Data from these cells has been included in the results above; this section refers to effects specifically related to crus stimulation.

Thirteen cells could be antidromicaly driven by the crus electrode. Eight cells were driven antidromically from crus and also showed an orthodromic response to striatal stimulation. Mean latencies were 10.46+/-9.15ms and 7.25+/-0.5ms respectively. Five cells were antidromically driven from both crus and striatum, with latencies of 2.16+/-1.68ms and 0.88+/-0.25ms (mean+/-sd) respectively. An example is shown in figure 2.13. The latencies of responses to crus stimulation of the two groups were not significantly different (figure 2.14(a)). The site of stimulation is indicated in figure 2.14(b).

The rates of spontaneous activity of all cells which were antidromically driven from the crus cerebri (mean +/- s.d. 29.4+/-23.8 spikes/second) were significantly faster than all other spontaneously active cells (13.8+/-11.6 spikes/second), with $p=0.003$ (figures 2.15, 2.16).

The mean rate of spontaneous activity of cells which were antidromically driven from both sites (17.0+/-18.4 spikes/second) was not different from that of the remaining general spontaneously-active cell population (15.9+/-14.6 spikes/second).

Other effects were seen such as inhibition from both electrodes; orthodromic stimulation from the striatum and inhibition from the crus; excitation following crus stimulation; mixed inhibition and excitation of spontaneous activity.

Apart from the antidromic activity, we rarely saw any activity with a timescale of milliseconds driven by the crus electrode (i.e. orthodromic), although long term changes (over several seconds) were sometimes seen, suggestive of polysynaptic influences, maybe via the striatum.

6-OHDA lesioned rats

Eleven units were recorded from the GP of three rats in which 6-hydroxydopamine lesions had been made at least 6 months earlier. The mean firing rate of spontaneously active cells was 25.8+/-13.3 impulses/second, range 6.7-48.2 (figure 2.17). This was not significantly different from the mean firing rate of spontaneously active cells in unlesioned animals. There was no apparent difference in firing patterns between lesioned

and unlesioned rats, as demonstrated by the interspike interval histograms.

The most distinctive feature of cells in lesioned animals is that some of them were markedly more responsive to striatal stimulation than cells recorded in unlesioned rats. Examples of post-stimulus histograms are shown in figure 2.18. A particularly striking example is shown in figure 2.18(e). This kind of multiple response was seen only in lesioned rats and never in control unlesioned animals.

Anatomical results

In each animal with a successfully placed striatal injection of either True Blue or Fluorogold retrogradely-labelled cell bodies were found in the GP. In the anterior portion of the nucleus the cells were located laterally, just within the pallidostriatal border. In the middle of GP cells were arranged both laterally and more medially within the nucleus. At more caudal levels within GP behind the anterior commissure (1mm caudal to bregma) the medial group of cells was still visible while neurons labelled in the lateral zone were scarce. There were usually no labelled cells in the entopeduncular nucleus, although we often found some cell bodies among the fibres of the internal capsule immediately caudal to GP, which were probably pallidal cells.

Figure 2.19 plots the results from a typical successful True Blue injection. When small injections of tracer were localised to the striatum, no retrogradely labelled pallidal cells were double labelled with ChAT. If the injection of the retrograde label was bigger and especially if it leaked to cortex, a wider distribution of labelled cell bodies was seen.

Discussion

Electrophysiological recordings were made within the globus pallidus from a variety of units with different characteristics of spontaneous activity and responses to striatal stimulation.

Spontaneous activity

The range of rates of spontaneously active cells (0.6-76.6 spikes/second) was different from that seen by other authors in rats (Bergstrom and Walters, 1981; Bergstrom *et al.*1984; Hirata and Mogenson, 1984). Only one third of units anatomically localised to GP met the criterion of the firing rate (10-70Hz) used by Bergstrom and co-workers (Bergstrom and Walters, 1981; Bergstrom *et al.*1984). This could have been due either to our halothane anaesthetic slowing down pallidal cells or to striatal stimulation activating units which would not otherwise have been noted.

Bergstrom and co-workers used chloral hydrate anaesthesia or paralysed and locally-anaesthetised rats, recording rates of 10-70 spikes/second. Hirata and Mogenson (1985) used urethane anaesthesia, and cite rates of 8-77 spikes/second. However, in another study using chloral hydrate in rats (Toan and Schultz, 1985), the rates of some pallidal cells in their figures are clearly firing at a rate of less than 10Hz.

None of these studies involved stimulation of the striatum, and thus they might not have seen the silent units which we recorded only as a result of striatal stimulation.

It is possible that the slower and silent cells could correspond to pallidocortical (putative cholinergic) neurons which have a wide range of reported spontaneous activity (Aston-Jones *et al.*1985; Reiner *et al.*1987), although this does not explain why other workers did not record the cells which were spontaneously active at a slow rate. Although we would not have been able to test the silent cells for antidromic activity we would expect that these cells would be antidromically driven from the stimulating electrode in cortex, or from their axons in striatum. However, the characteristics of the activity evoked from the silent cells were significantly different from those of the antidromically-driven cells. This does not rule out the possibility that some of the

silent cells were pallidocortical cells, but as a group their longer and more variable latencies would suggest otherwise (see below).

The spontaneously active antidromically-driven cells might have been pallidocortical cells, but if so, they were not distinguished by slower rates of firing.

There was no specific anatomical localisation of either antidromic or slower cells; Divac (1975) and Lehman *et al.* (1980) showed the cholinergic cortically-projecting cells in the rat to be situated in the ventromedial region of GP. Ingham *et al.* (1985) found that although these cells tended to be located in the peripheral areas of GP, they could be found within the middle of the nucleus, although in another study (Ingham *et al.*1988) the cells labelled retrogradely from a cortical injection were always situated in the ventral and lateral margins of GP.

Orthodromic activity in pallidal units

The units recorded from GP which responded to striatal stimulation with an orthodromic spike fell into two groups, depending on whether they showed spontaneous activity. The latency of the spike in the spontaneously active group was significantly longer than that for the silent units. The variance of the latencies of the spontaneously active cells was significantly larger than

for the silent group. This suggests that the input from the striatum to the active cells is both longer and less direct than that to the silent cells, i.e. that it is polysynaptic.

The silent units might have been striatal efferent fibres; striatal cells are known to have a low level of spontaneous activity from studies in the rat and cat (Bloom *et al.*1965; Purpura and Malliani, 1967; Sugimori *et al.*1978; Calabresi *et al.*1990). They sometimes responded to striatal stimulation with a burst of spikes, which is a characteristic of striatal cells. This is different from the responses of pallidal cells which intracellular recordings have shown to be a hyperpolarisation of the cell membrane and an inhibition of spontaneous firing (Park *et al.*1982), sometimes followed by a rebound excitation (Nakanishi *et al.*1985). Often a series of silent units with similar latencies were recorded in close succession; it is tempting to speculate that this phenomenon might have been due to the passage of the recording electrode through a fibre bundle.

Striatal stimulation produced inhibition, excitation or a mixed response in many of the spontaneously-active GP cells. This is in accordance with other observations (Park *et al.*1982; Nakanishi *et al.*1985).

Pallidostriatal cells?

Twenty-three cells were antidromically driven from the striatum. They were characterised by a short latency spike which varied very little. There was no difference in the rates of spontaneous activity or in the location of these cells which would distinguish them from the other cells.

These cells have not been previously described electrophysiologically, so it is important that all alternative explanations for these observations be excluded.

The main alternative explanation is that these were pallidocortical cells. Stimulation by an electrode located in the striatum would activate axons passing through it from the site of the recording electrode to the cortex. Cells which are outliers of the nucleus basalis of Meynert have been described within the pallidum. They are known to be cholinergic (Kievit and Kuypers, 1975; Lehmann *et al.*1980; Mesulam *et al.*1983). The anatomical studies presented here identify a population of retrogradely-labelled cells from the striatum which were not cholinergic. The locations from which the pallidostriatal activity was recorded were throughout the pallidum. Although most studies of cortically-projecting cells in this part of the brain find them to be situated around the periphery of GP, or within the fibres of the internal capsule (Divac, 1975;

Lehmann *et al.*1980; Ingham *et al.*1988), cholinergic pallidocortical cells have been described in the middle of the nucleus (Ingham *et al.*1985).

We did see a few cells which were double labelled for retrograde tracer and ChAT in the middle of the GP, but it is highly unlikely that these could have accounted for all the antidromically-driven cells which were seen. Van der Kooy and Kolb (1985) identified a group of small GP cells projecting to the cortex in rats. Their cells did not have collaterals to the midbrain and thus they are distinct from a number of our cells which responded antidromically to stimulation of the crus cerebri (see below).

Other authors have demonstrated a pallidostriatal pathway using anatomical techniques (Staines *et al.*1981; Jayaraman, 1983; Staines and Fibiger, 1984). The cells of this tract are probably GABAergic, as the majority of pallidal cells contain GABA; GABAergic pallidostriatal terminals have recently been directly demonstrated by Staines and Hincke (1991).

What might be the significance of a GABAergic pallidostriatal pathway? There are a number of reciprocal pathways within the basal ganglia, such as the striatonigral and nigrostriatal pathways, and between the GP/LGP and the subthalamic nucleus. This

would suggest that feedback loops are an important part of the regulation of neuronal activity in the basal ganglia.

The GP receives most of its input from the matrix compartment of the striatum (Graybiel *et al.*1979; Jimenez-Castellanos and Graybiel, 1989), via a GABAergic and enkephalinergic pathway, which has an inhibitory effect upon pallidal cells. If these terminals make contact with pallidostriatal cells, as is likely, given the size of the striatopallidal input, these cells will be inhibited. An increase in the activity of this input would result in a reduction of the inhibitory effect of the GP over the striatum.

Staines and Hincke (1991) have demonstrated GABAergic terminals from rat GP making contact with somatostatin-positive cell bodies in striatum. These cells make up one population of striatal interneurons, which are characteristically distributed throughout the striatum, in the vicinity of the boundaries between the two striatal compartments (see page 106 Chapter 3). They could play a role in communicating between the two compartments, although the terminals of these cells are confined for the most part to the matrix (Graybiel *et al.*1981; Gerfen *et al.*1985). The activation of striatal efferents to LGP would result in the disinhibition of these striatal interneurons. This might be modified by other influences of the GP, such as the glutamatergic

input from the STN. The somatostatin interneurons make contact with medium spiny cells (DiFiglia and Aronin, 1984), although the effect of somatostatin as a neurotransmitter in striatum is not known. (Both excitatory and inhibitory effects have been demonstrated upon various other preparations (Kelly, 1982).)

When the pallido-striatal input is removed, in animals with lesions of GP, there is a loss of staining in these interneurons (Staines and Hincke, 1991). Although these cells are known to use the electrophysiologically inhibitory GABA as a neurotransmitter, some other factor from this pathway could have regulatory effect upon peptide synthesis.

In Huntington's disease the NPY/somatostatin/NAPDH-diaphorase positive interneurons are selectively preserved (Ferrante *et al.*1985). The cells of the striato-LGP pathway are among the first to be lost; these are also located primarily in the matrix. The decreased inhibitory influence upon the LGP (Ellison *et al.*1987; Reiner *et al.*1988) would result in increased activity of pallidostriatal cells. The increased input from the LGP-striatal pathway could result in the increase in synthesis of NO by the somatostatin/NPY/NADPH-diaphorase interneurons, with ensuing toxicity to the surrounding cells in the matrix (Dawson *et al.*1991a,b). Some of these project to LGP,

and as they are destroyed the situation becomes exacerbated. What is not known, however, is what initially triggers this destructive cycle, but this may soon be discovered from studies of the molecular biology of Huntington's disease.

The somatostatin interneurons also seem to be dependent upon an intact input from the dopaminergic nigral cells (Kerkerian-Le Goff *et al.*1991). Paradoxically, in animal models of Parkinson's Disease similar changes are seen as in HD, i.e. there is an increase in staining for NPY/somatostatin/NADPH-diaphorase. However, a loss of staining of NPY only was observed when dopaminergic transmission was interrupted by administration of alpha-methyl-para-tyrosine or haloperidol (Kerkerian-Le Goff *et al.*1991).

This would suggest that the regulation of production of somatostatin/NPY/NADPH-diaphorase is affected in a complex manner by inputs to the interneurons. These cells, which receive inputs from the pallidostriatal cells, reflect the effects of different disease states upon the basal ganglia.

Antidromic driving from the crus cerebri

Thirteen cells were antidromically driven from the crus cerebri. Four of these were orthodromically driven from the striatum, and in some cases the antidromic crus spike was shown to collide with the striatal spike, thus

demonstrating that these were striatopallidofugal cells, maybe to the substantia nigra. The other cells showed varying responses to striatal stimulation; an inhibitory response might have been an indicator of a pallidonigral cell, receiving a GABAergic input from striatum.

The cells which were antidromically driven from the crus fired significantly faster than other pallidal cells, although the range (0-76.6 spikes/second) was similar (general population 0-50). This would suggest that these cells make up a distinct group, some of them - which were antidromically driven from both sites - being apparently pallidonigral with collaterals to striatum, and others not. The five cells recorded which were antidromically driven from both sites were not significantly different in terms of their spontaneous firing rates.

The sites to which the GP is known to project in the midbrain are the subthalamic nucleus (McBride and Larsen, 1980; van der Kooy *et al.*1981) and the substantia nigra (Grofova, 1975; Hattori *et al.*1975; Bunney and Aghajanian, 1976; Kim *et al.*1976; Larsen and Sutin, 1978; McBride and Larsen, 1980).

It is not likely that these were pallidosubthalamic cells, even though the location of the crus cerebri stimulating electrode was such that it could have

stimulated the subthalamic nucleus. There is a major reciprocal connection between the GP and the subthalamic nucleus, and in this situation a significant excitatory orthodromic response would have been seen. This was not the case, suggesting that the subthalamic nucleus was not being stimulated. Also, striatal collaterals of pallidosubthalamic neurons have not (yet) been described.

It seems likely that the pallidal cells which responded antidromically to electrical stimulation of both the crus cerebri and the striatum correspond to the cells seen by Staines and Fibiger (1984) using anatomical techniques; and which project to topographically equivalent areas of SN and striatum.

Other effects of crus stimulation on GP cells

Short latency orthodromic activation of pallidal cells from the crus cerebri were seen rarely, although there is known to be a small input from SN to GP (Lindvall and Bjorklund, 1979). Longer latency effects were far more common, and were excitatory, inhibitory or mixed.

The relatively low numbers of neurons showing antidromic activation from the striatum and the crus is undoubtedly due to the topographical organisation which the pallidostriatal and pallidonigral pathways share with many other connections between nuclei of the basal ganglia. Staines and Fibiger (1984) suggest that the

pallidostriatal, pallidonigral and striatonigral pathways are in register, so that pallidal cells project to both the origin and termination of a topographically designated group of striatonigral neurons. Smith and Bolam (1991) have described convergence of the pallidonigral and striatonigral systems upon nigral efferent cells.

The neurochemistry of the pallidostriatal pathway is not yet known, but the majority of pallidal cells, and of previously-described pallidal efferents, contain GABA. The pallidonigral pathway, which is at least in part a collateral of the pallidostriatal pathway, as shown here, is GABAergic (Smith and Bolam, 1989). The present data, along with previous investigations (Arbuthnott *et al.*1983) confirm that the pallidostriatal cells do not contain substance P, enkephalin or acetylcholine.

Lesioned animals

In rats with chronic 6-OHDA lesions of the substantia nigra the mean firing rate was significantly increased, but only when all cells, including silent ones, were included. When the rates of spontaneously active cells were compared there was no difference between the two groups. This suggests that some of the previously silent cells were activated in the absence of dopamine. If the silent units were striatal efferents, then it is possible that they would be more active in the absence

of dopamine.

An increase in the activity of striatal efferents in rat and primate models of PD has been demonstrated by metabolic studies (Wooten and Collins, 1983; Crossman *et al.*1985; Mitchell *et al.*1986) and electrophysiological studies (Schultz and Ungerstedt, 1978a; Arbuthnott *et al.*1987). However, these changes may only be for a short period following the lesion. The metabolic studies were all done within a few weeks post-lesion. These studies show an increase in the uptake of carbon 14-labelled 2-deoxy-D-glucose (Sokoloff *et al.*1977), and are interpreted as demonstrating an increase in terminal activity (Mata *et al.*1980).

Schultz and Ungerstedt (1978a) identified their striatal cells by cortical stimulation in choral hydrate-anaesthetised rats, and found that the very slow rates of spontaneous activity (median 0.04 impulses/second) were increased in the short term (median 0.28 impulses/second), but reverted to slower rates in the long term. They do not mention any cells which were completely silent, and identifiable only by stimulation, although we may have missed very slow firing rates, or the cells might have been silent under the different anaesthetic.

Previous investigations in the rat of the effects upon GP cells of altering dopaminergic stimulation to the

striatum, have usually studied short term effects (Bergstrom and Walters, 1981; Bergstrom *et al.*1984; Toan and Schultz, 1985). In primate models of Parkinson's disease, the recordings have usually been carried out within a few weeks of the lesion. In primates with electrolytic lesions of the ventral tegmental area, following reserpine treatment (Filion, 1979), or using MPTP treatment (Miller and Delong, 1987), there was no change in the mean rate of LGP cells, although the firing pattern became more regular and less bursting.

The adaptation to the lesion may explain why we did not observe any striking differences in the firing rates and patterns of spontaneously active GP cells, apart from as discussed above.

However, we did see a striking feature of cells from lesioned animals following electrical stimulation of the striatum. The cells in our small sample were strikingly hyperresponsive to striatal stimulation. The remarkable similarity between figure 2.18(e) and the illustration of the response of GP neurons to striatal stimulation *in vitro* (Nakanishi *et al.*1985) suggests that the "hyperresponsive" cells lack an important influence of dopamine which is also lost *in vitro* (although obviously the time course is different here). Similar oscillatory responses, recorded from MGP cells, were seen in MPTP-treated monkeys following striatal stimulation (Filion

*et al.*1989) which the authors suggest may be related to the production of tremor in Parkinson's disease.

Interestingly, chronic striatal lesions in cats (Sachdev *et al.*1989) resulted in an increase in GP neuron discharge rate, as would be predicted with the removal of a GABAergic input, but similarly to the primate SN lesions, there was also a decrease in bursting activity. This could be explained if both GABA and dopamine were necessary for this pattern of firing, but more studies would be needed to confirm this.

Various authors in different types of studies have shown that dopamine in the striatum has an effect which can be best descibed as modulating or focussing inputs (Hirata *et al.*1984; Hirata and Mogenson, 1984; Toan and Schultz, 1985; Chiodo and Berger, 1986; Girault *et al.*1986) which is reflected in the activity of the pallidum. Filion *et al.*(1989) see the hyperresponsiveness of MGP cells to striatal stimulation as a loss of selectivity in the absence of dopamine. This is reflected by a loss of specificity of response to passive limb movements (Filion *et al.*1986, 1988). In our experiments the inability of the pallidum to process striatal inputs in the absence of dopamine, is reflected by marked oscillations, which may be mediated by striatal GABAergic recurrent collaterals (Girault *et al.*1986; Filion *et al.*1989).

Summary

The experiments described above present anatomical and electrophysiological evidence for a pallidostriatal pathway in the rat. Some of the cells observed electrophysiologically also had branches to the midbrain.

The activity seen in GP cells following chronic 6-hydroxydopamine lesions correlate with other studies, and are undoubtedly due to the loss of dopaminergic modulation of striatal cells.

The globus pallidus and the striatum are closely related anatomically and functionally, with lesioned states reflecting the imbalance of neuronal activity seen in human disorders of movement.

Figure 2.1 Distribution of rates of spontaneous activity of all pallidal cells

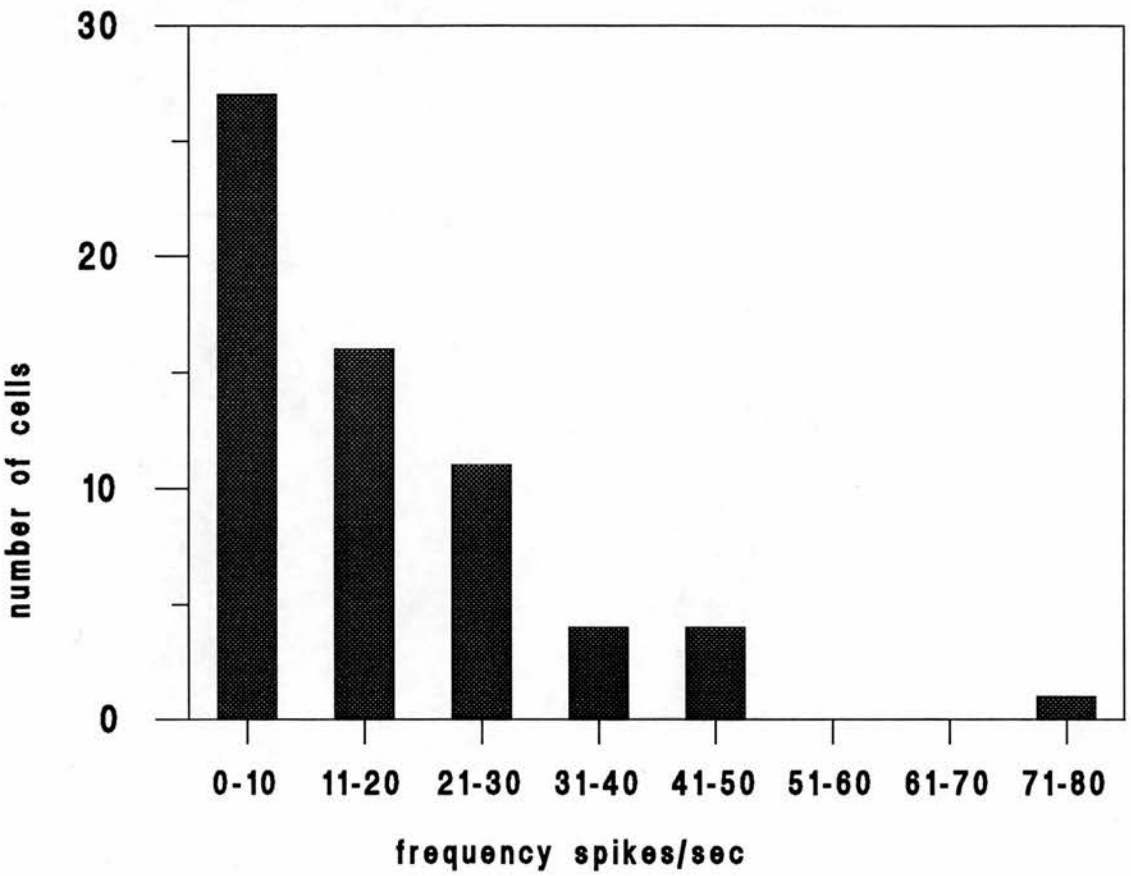


Figure 2.2 Coronal section through rat brain at 7.70mm rostral to the interaural line. Symbols in the globus pallidus (GP) indicate the positions of the15 slowest (▽) and the 15 fastest (●) firing
spontaneously active cells; these slowest cells fired at a rate of less than 5 impulses per second, the fastest fired at a rate of more than 24 impulses per second

CP=caudate nucleus/putamen, GP= globus pallidus, IC=internal capsule, VP=ventral pallidum

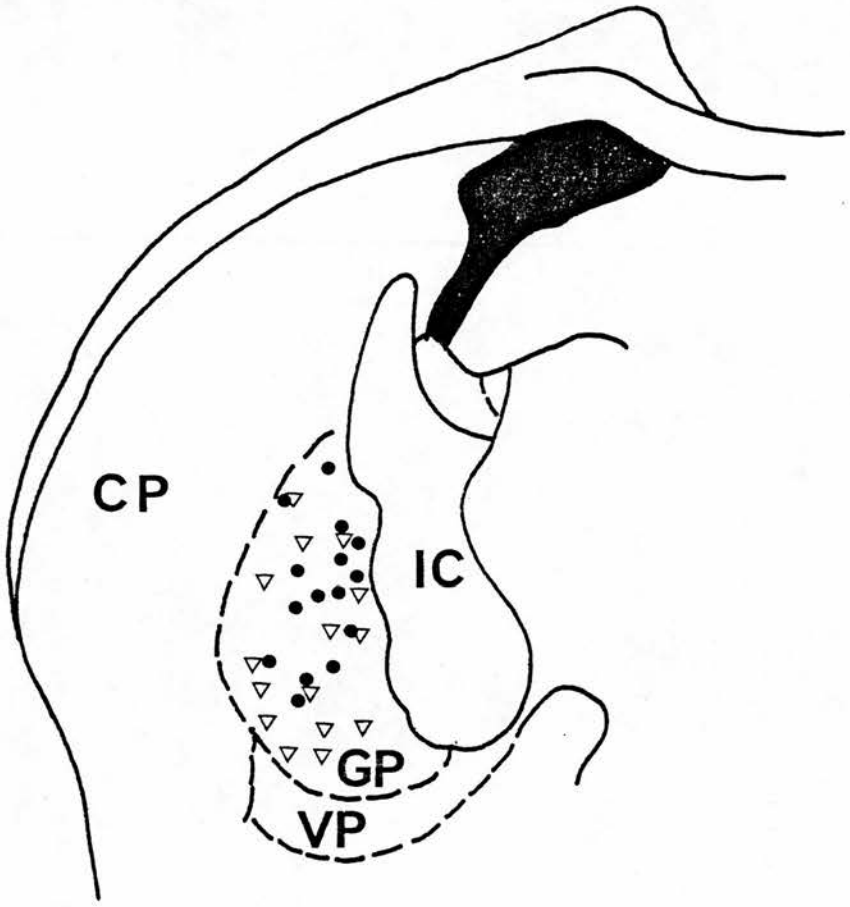


Figure 2.3 Photograph of oscilloscope screen demonstrating the response of a spontaneously active pallidal cell to striatal stimulation (7mAx8ms) at the time indicated by the arrow; cell1010, 200 sweeps

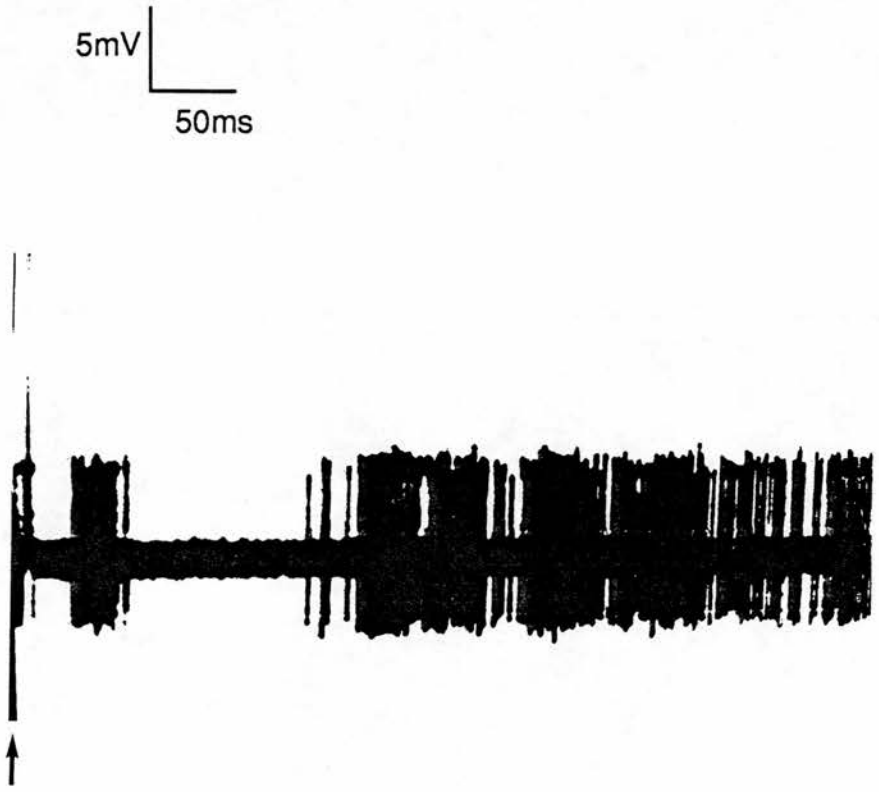


Figure 2.4 Coronal section through rat brain at 8.2mm rostral to the interaural line, indicating electrode track through the globus pallidus. Post-stimulus histograms from five cells are given, showing the responses of these cellls to striatal stimluation, at the time indicated by the arrow, with the positions of cells marked

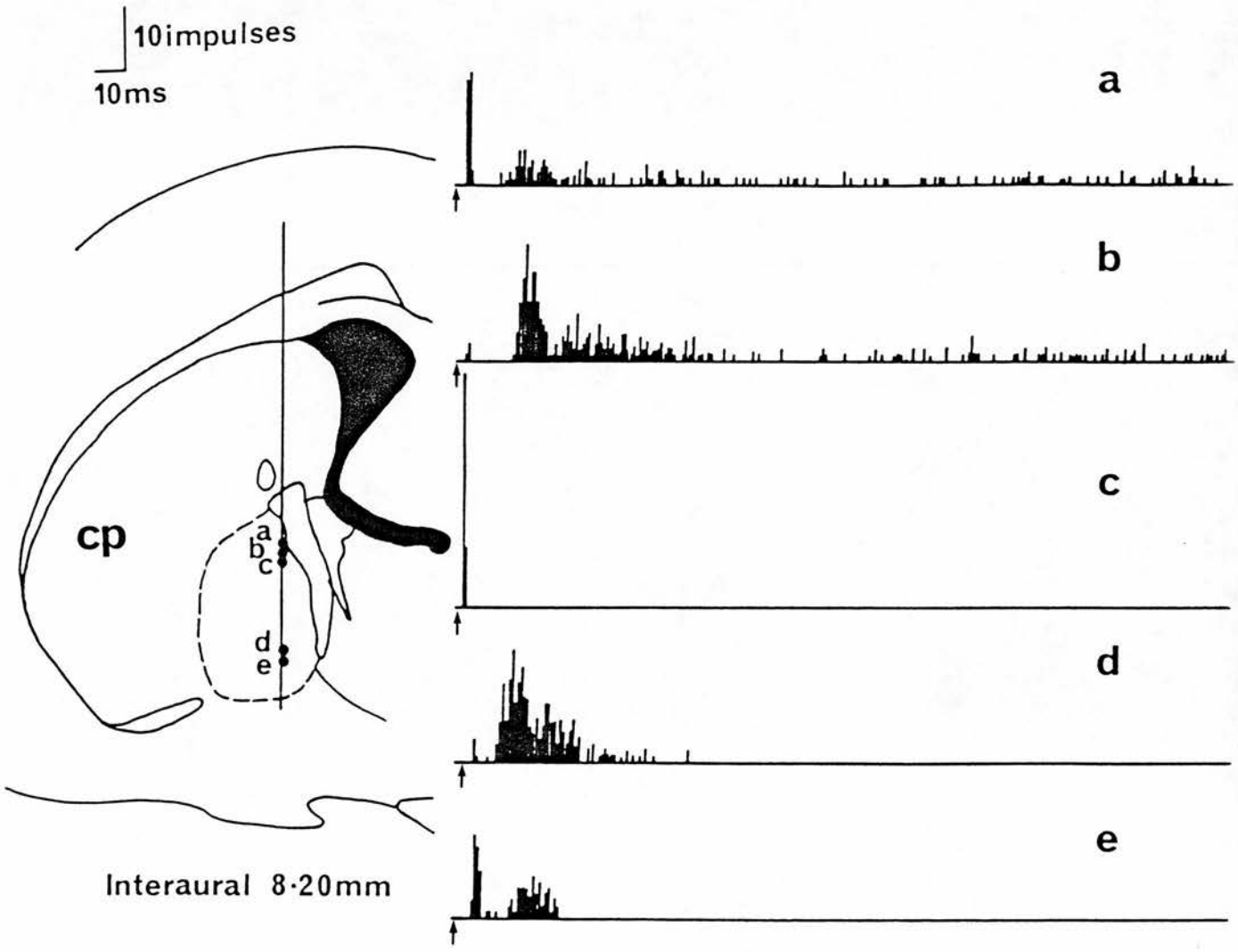


Figure 2.5 Distribution of the rates of spontaneous activity of pallidal cells, classified by response to striatal stimulation as orthodromically driven, antidromically driven, or not driven

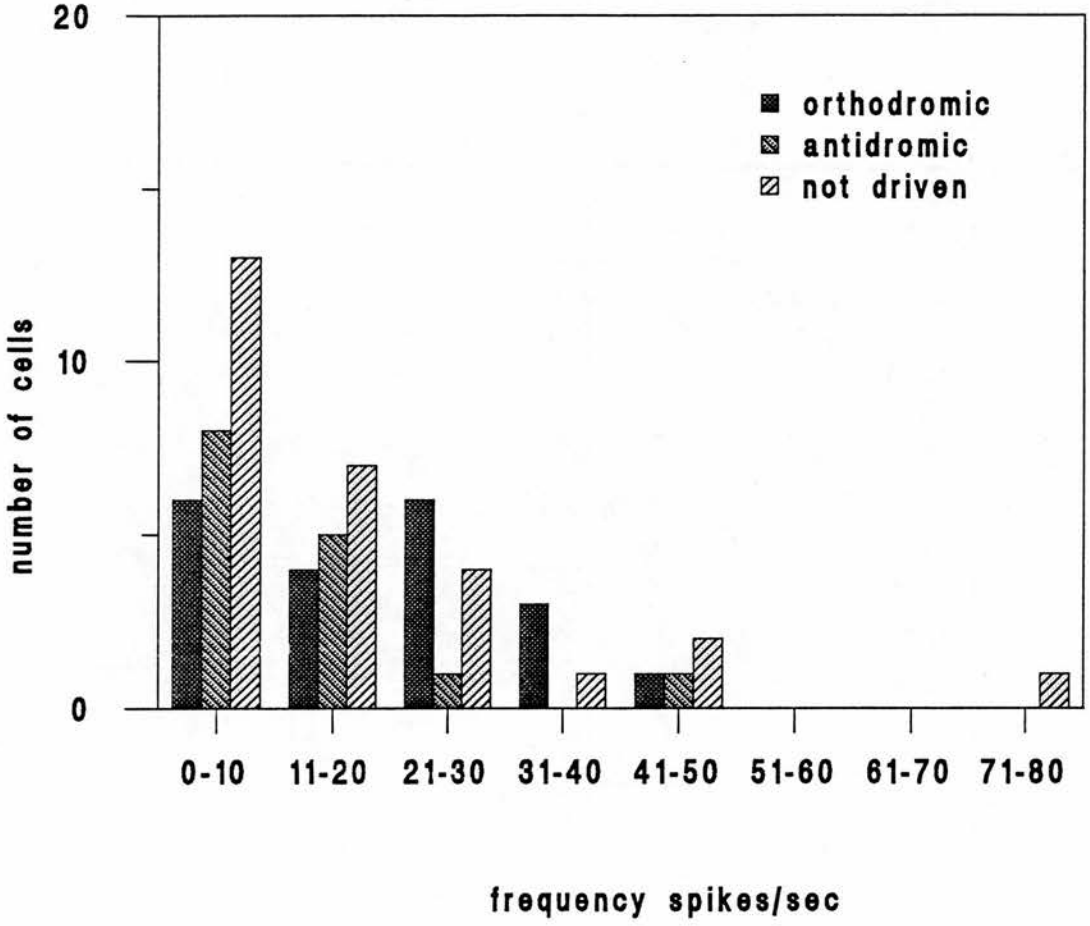


Figure 2.6(a) Burst response to striatal stimulation (300μAx2ms), indicated by arrow, of cell 6007; 5ms/division, 10mv/division

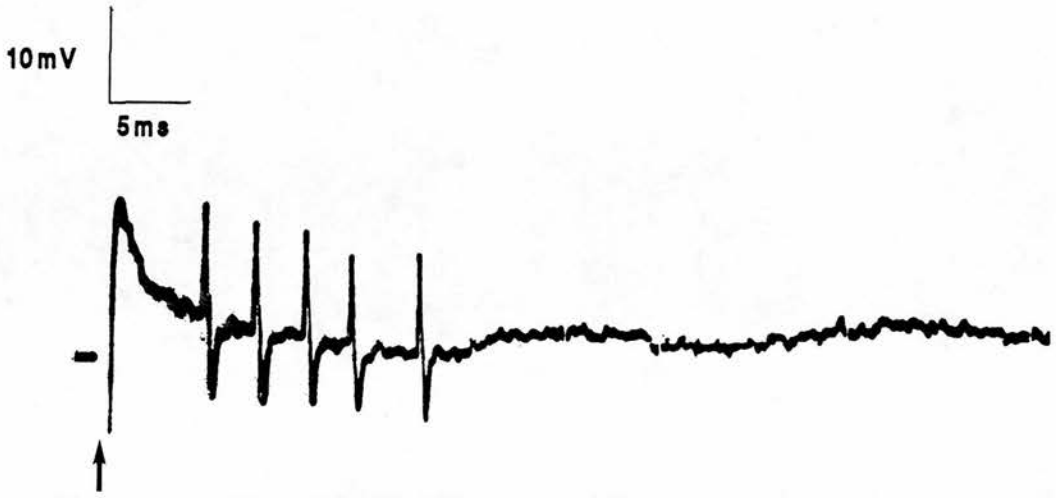


Figure 2.6(b) Number of orthodromically driven spikes in response to striatal stimulation, comparing spontaneously active cells with silent cells

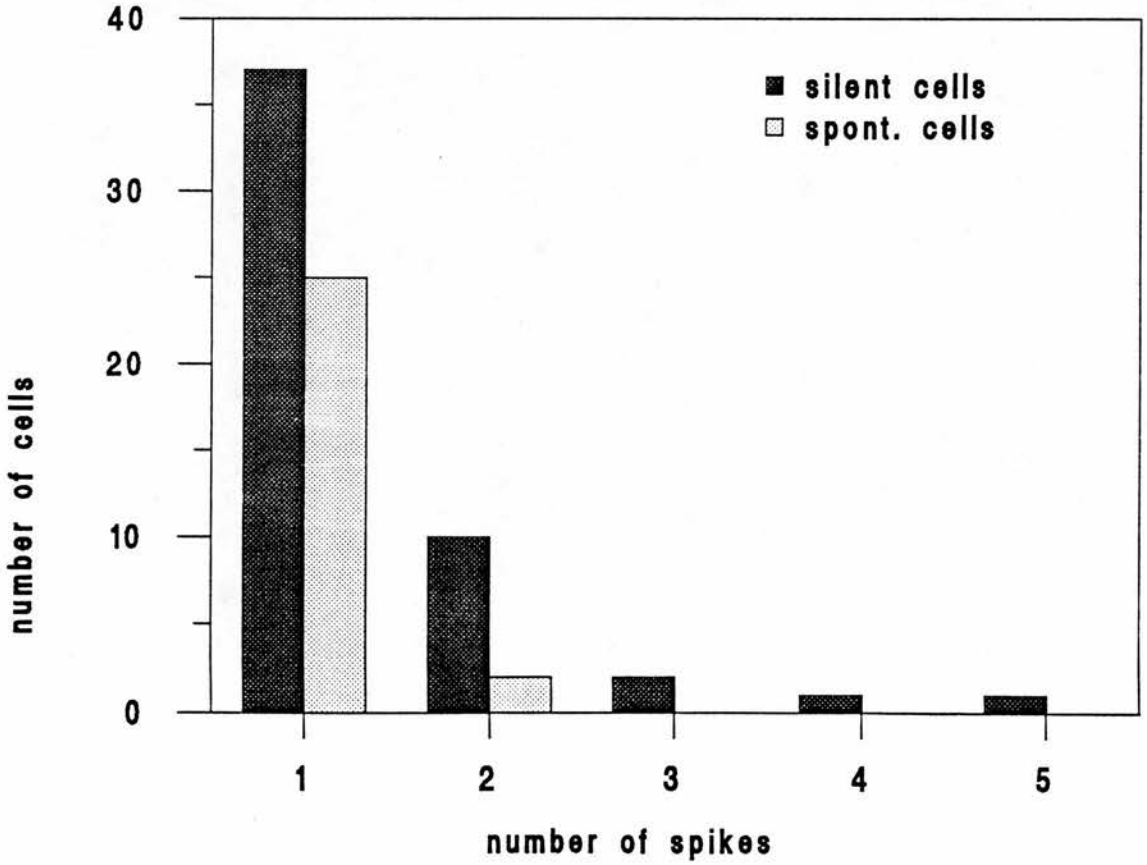


Figure 2.7 Distribution of latencies of first orthodromically-driven spike recorded in the globus pallidus following striatal stimulation, cells classified as spontaneously active or silent: (a) 20ms bins (b) 1ms bins, outlier at 180ms excluded

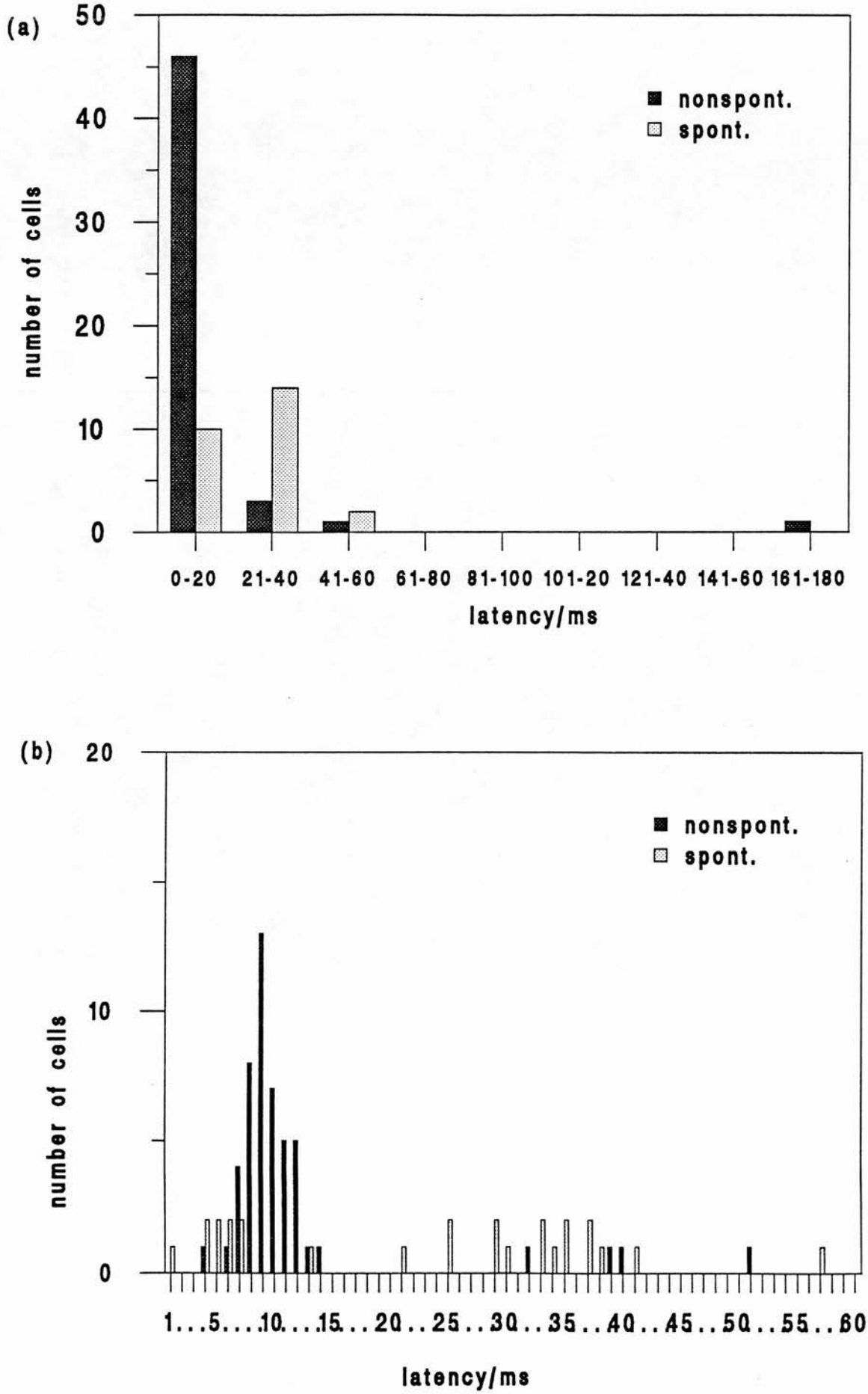


Figure 2.8 Histogram of the mean+standard deviation of latencies of pallidal cells orthodromically driven from the striatum, comparing spontaneously active versus silent cells. The mean latency of the silent cells is significantly smaller, p=0.0002.

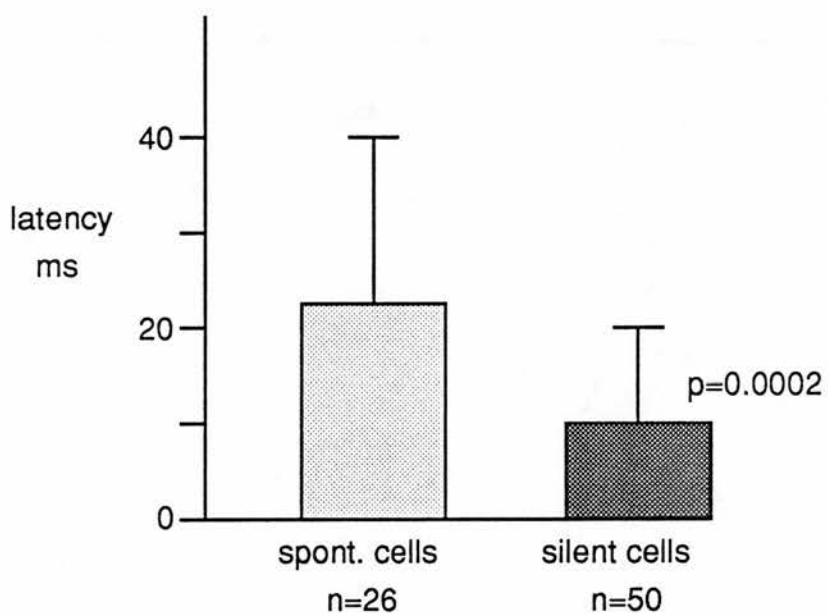


Figure 2.9 Collision tests demonstrating antidromic driving of two pallidal cells from the striatum; stimulation at the arrows, spontaneous activity indicated by asterisks
(a) evoked spike; collision; evoked spike
(b) evoked spike, several sweeps superimposed, demonstrating constant latency; collision following spontaneous spike

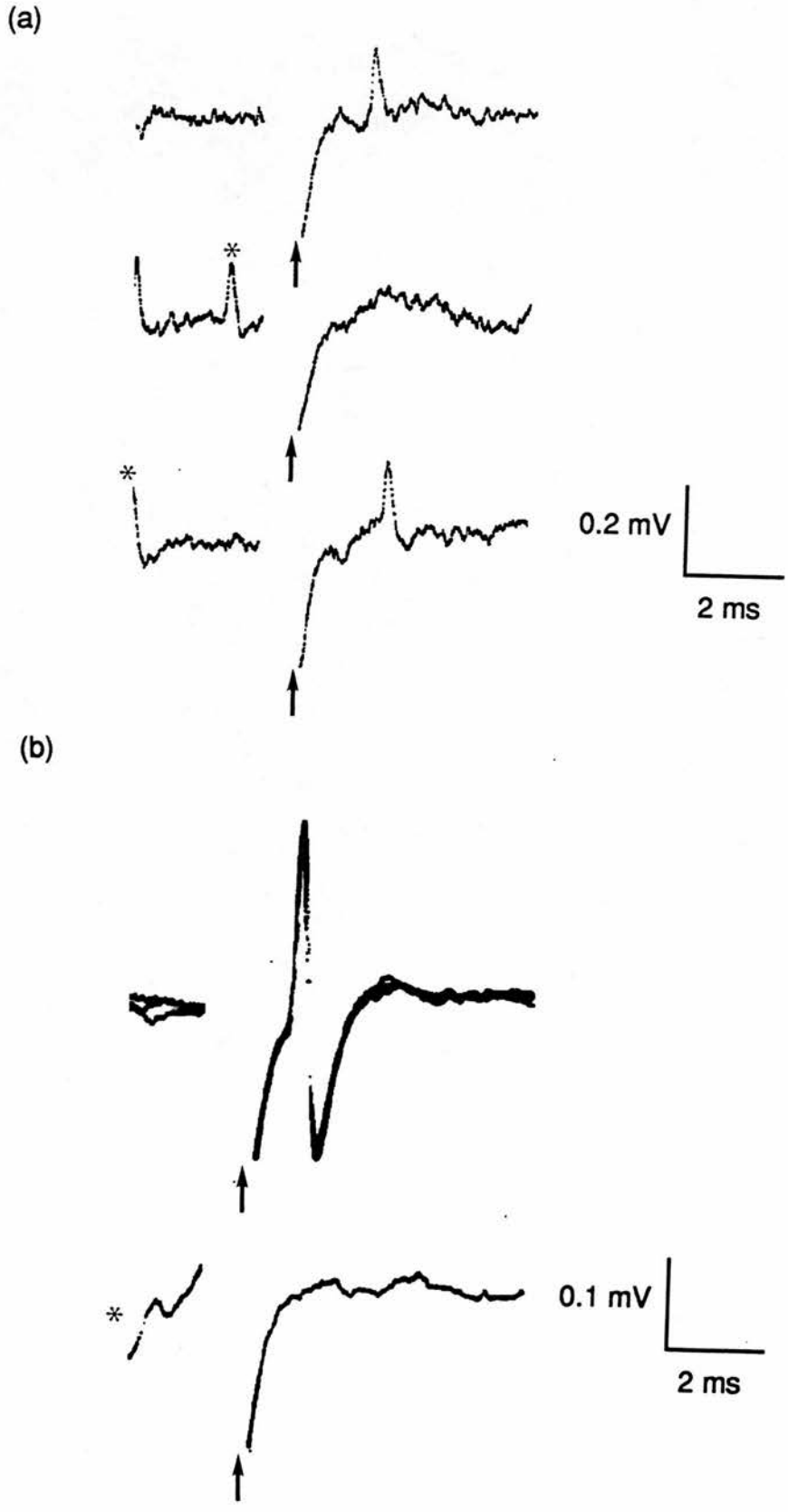


Figure 2.10 Collision test of pallidal cell1009; superimposed oscilloscope traces show striatal stimulation (2mAx1ms), as indicated by the arrows, triggered at different time intervals following the spontaneous activity (indicated by the asterisk). Collision is seen by the absence of a spike following the stimulus at 7ms after the spontaneous spike, but not at 8ms or 9ms latency, where a driven spike follows striatal stimulation.

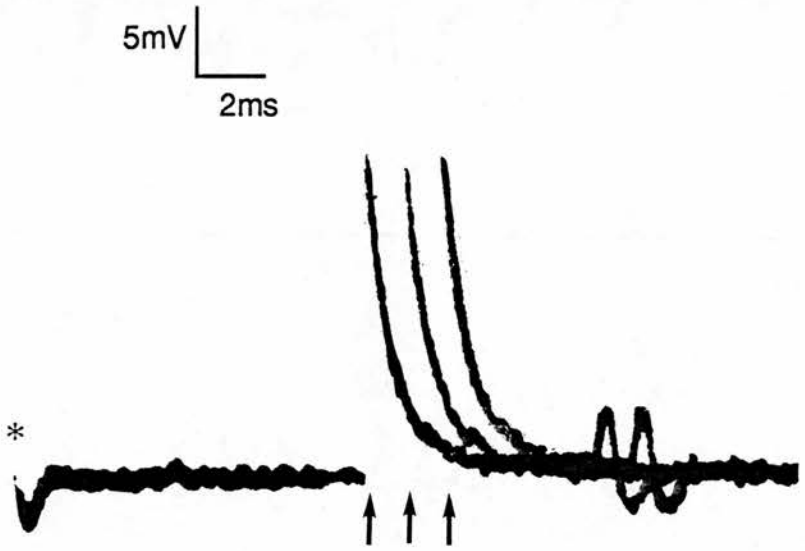


Figure 2.11(a) Distribution of the latencies of activity following striatal stimulation; cells classified as orthodromically driven - spontaneously active or silent, or as antidromically driven

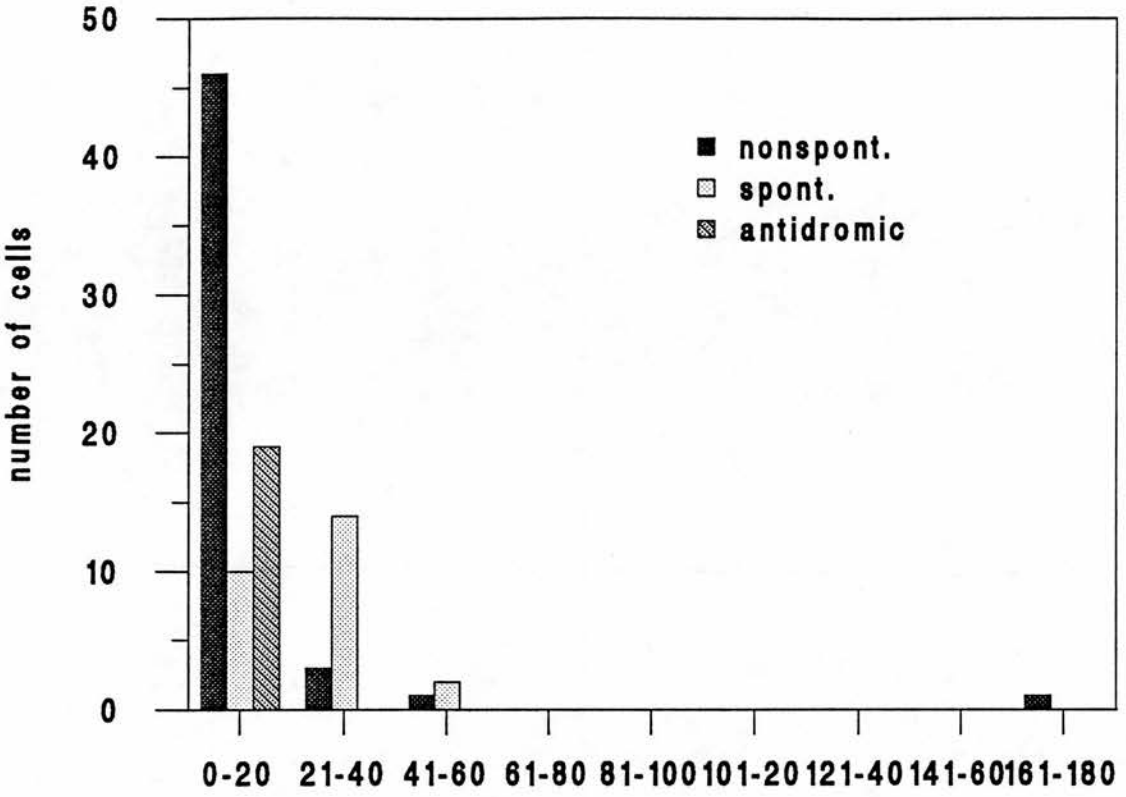


Figure 2.11(b) Distribution of latencies of activity following striatal stimulation - antidromic cells only

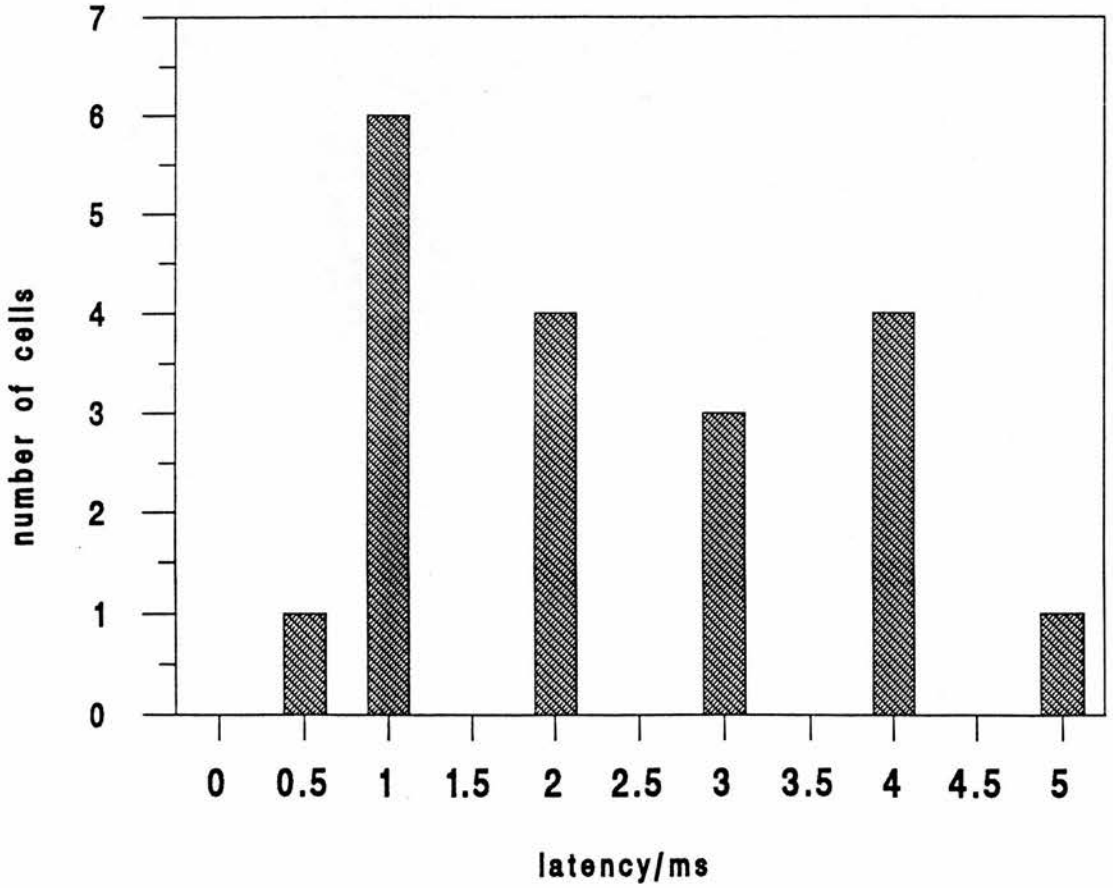


Figure 2.12(a) Histogram of mean+standard deviation of spike latencies following striatal stimulation, comparing all orthodromically driven cells (both silent and spontaneously active) with antidromically driven cells

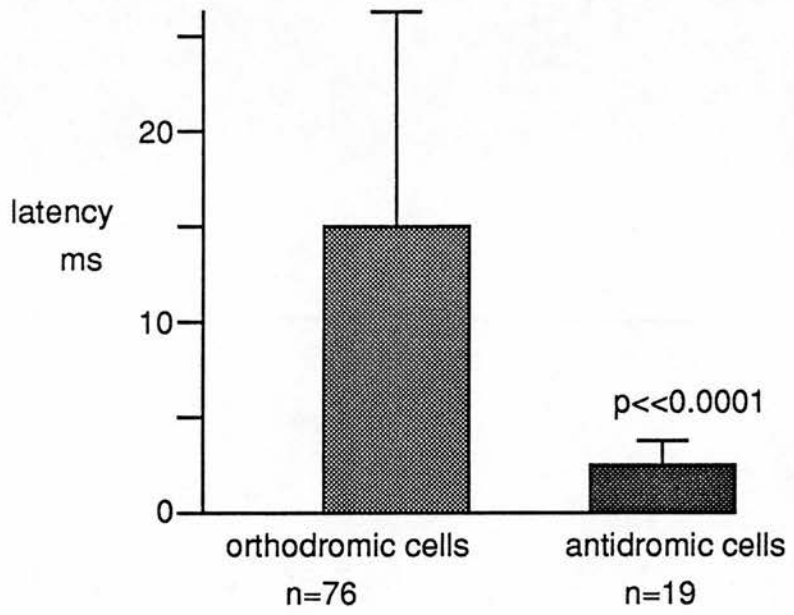


Figure 2.12(b) Histogram of mean+standard deviation of latencies comparing silent orthodromically driven cells with antidromically driven cells

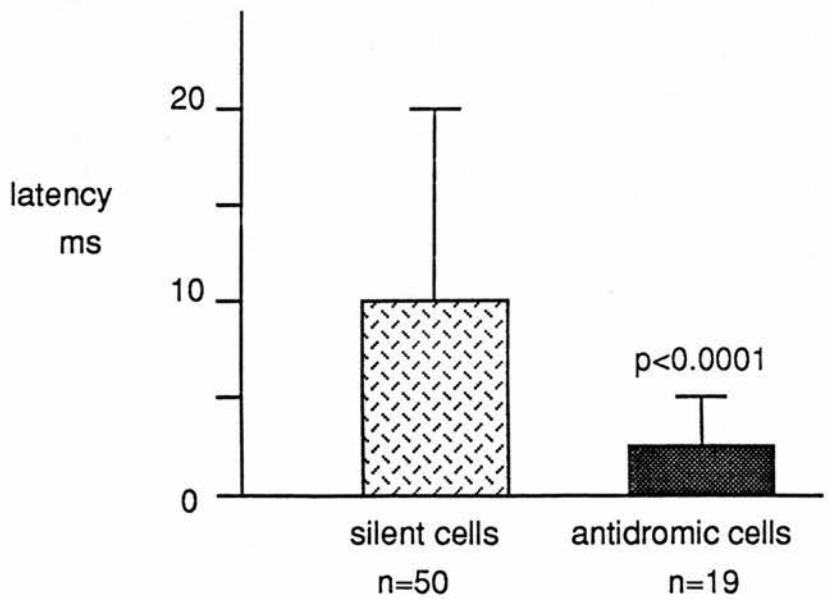


Figure 2.13 Oscilloscope records from a single pallidal cell
(a) spontaneous activity (several superimposed traces)
(b) superimposed traces of responses to striatal (80μAx0.02ms) and crus stimulation (300μAx0.02ms) at arrow ; note constant latency
(c) striatal simulation (arrows) at different latencies following spontaneous activity (at asterisk); the response to the earliest stimulus has collided (large arrow)
(d) stimulation of the crus cerebri, as (c)

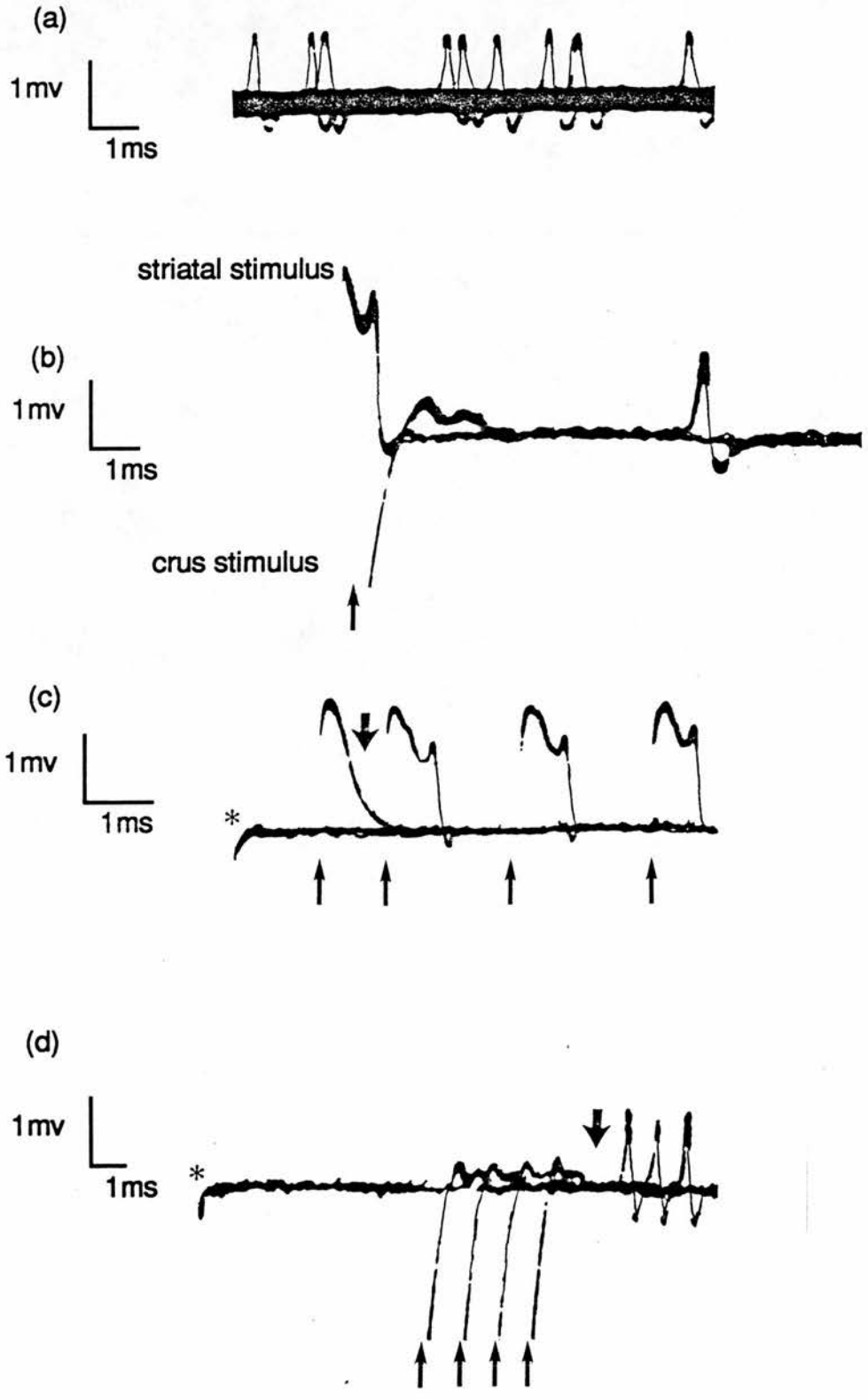
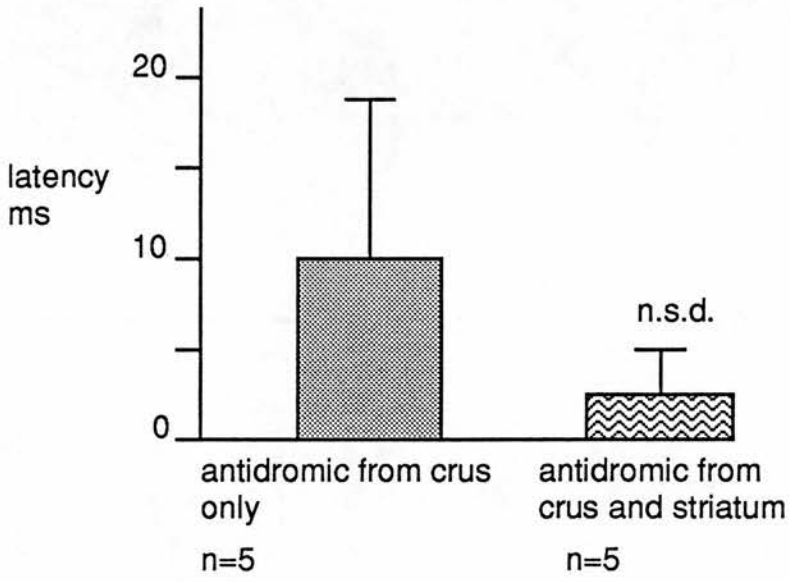


Figure 2.14(a) Histogram of mean+standard deviation of latency of spike from stimulation of the crus cerebri, comparing pallidal cells driven antidromically from the crus only, with those driven antidromically from both striatum and crus.



(b) Position of the stimulating electrode in the crus cerebri; coronal section at 4.7mm rostral to the interaural level

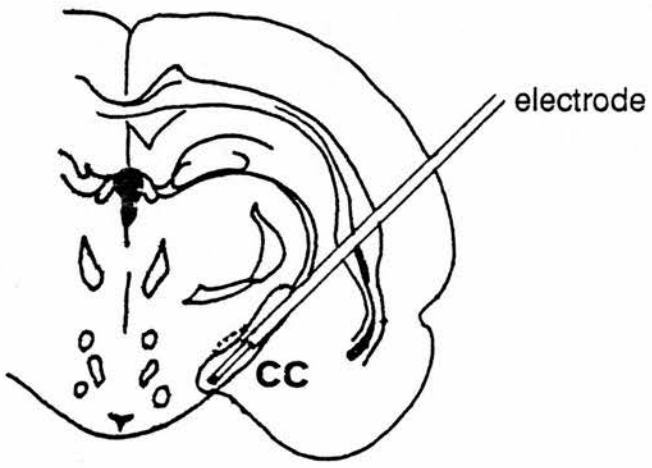


Figure 2.15 Distribution of rates of spontaneous activity for cells antidromically driven by stimulation of the crus cerebri (ad from crus) compared with all other cells (not ad from crus)

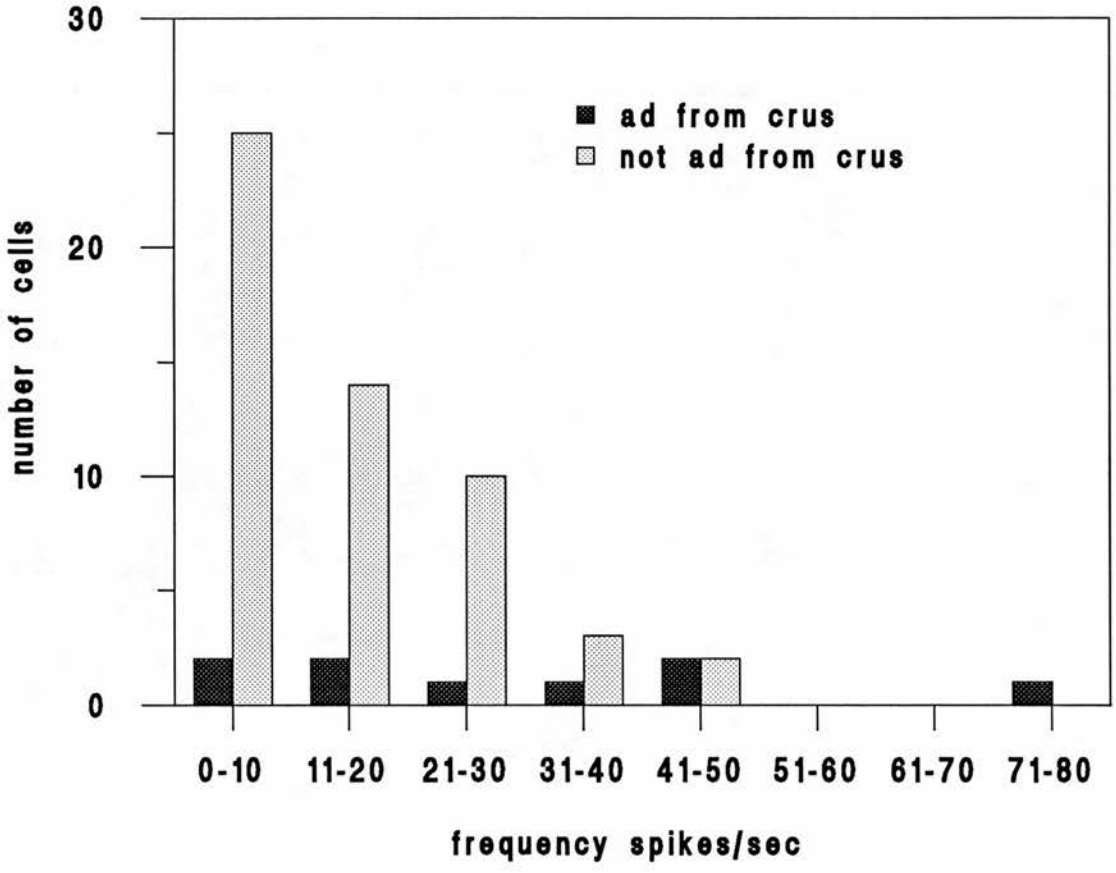


Figure 2.16 Histogram of the mean+standard deviation of rates of spontaneous activity of cells antidromically driven from the crus cerebri compared with all other cells

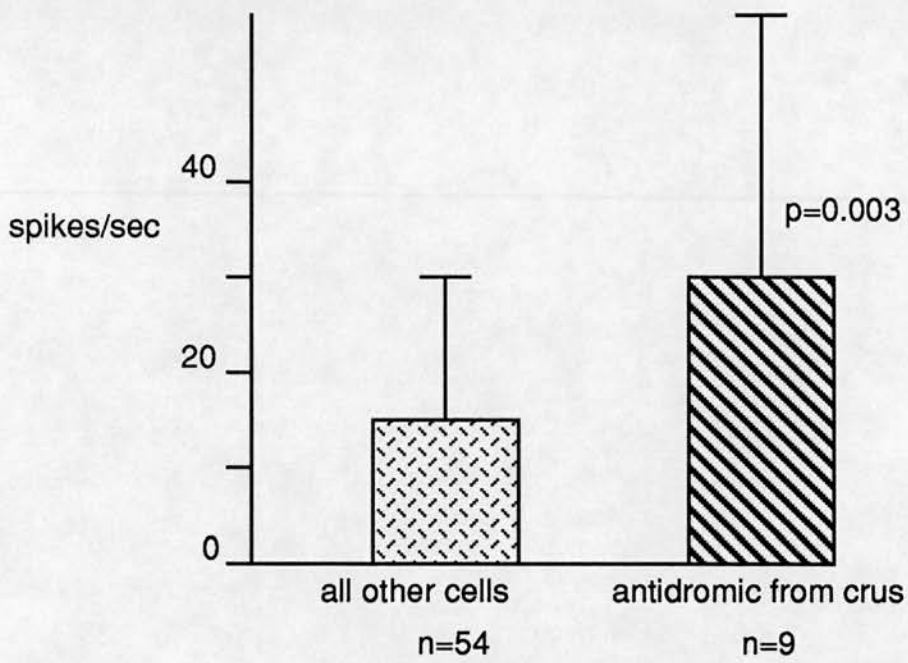


Figure 2.17 Distribution of rates of spontaneous activity of pallidal units recorded from 6-hydroxydopamine-lesioned rats (lesioned) compared with rates for cells from unlesioned animals (all spont. cells: unlesioned)

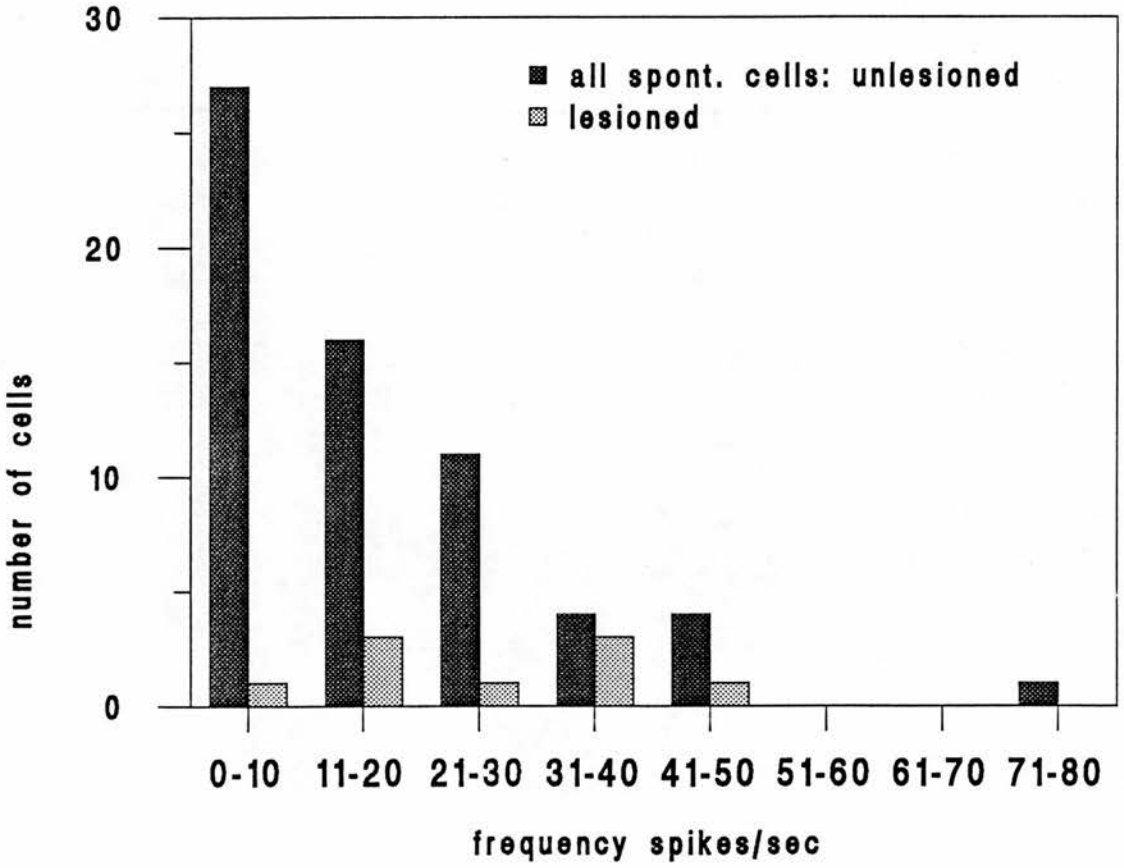


Figure 2.18 Post-stimulus histograms of pallidal cells from 6-hydroxydopamine-lesioned rats. Stimulation in the striatum, as indicated by the arrows; 50 sweeps; note that a different scale applies to part (e)

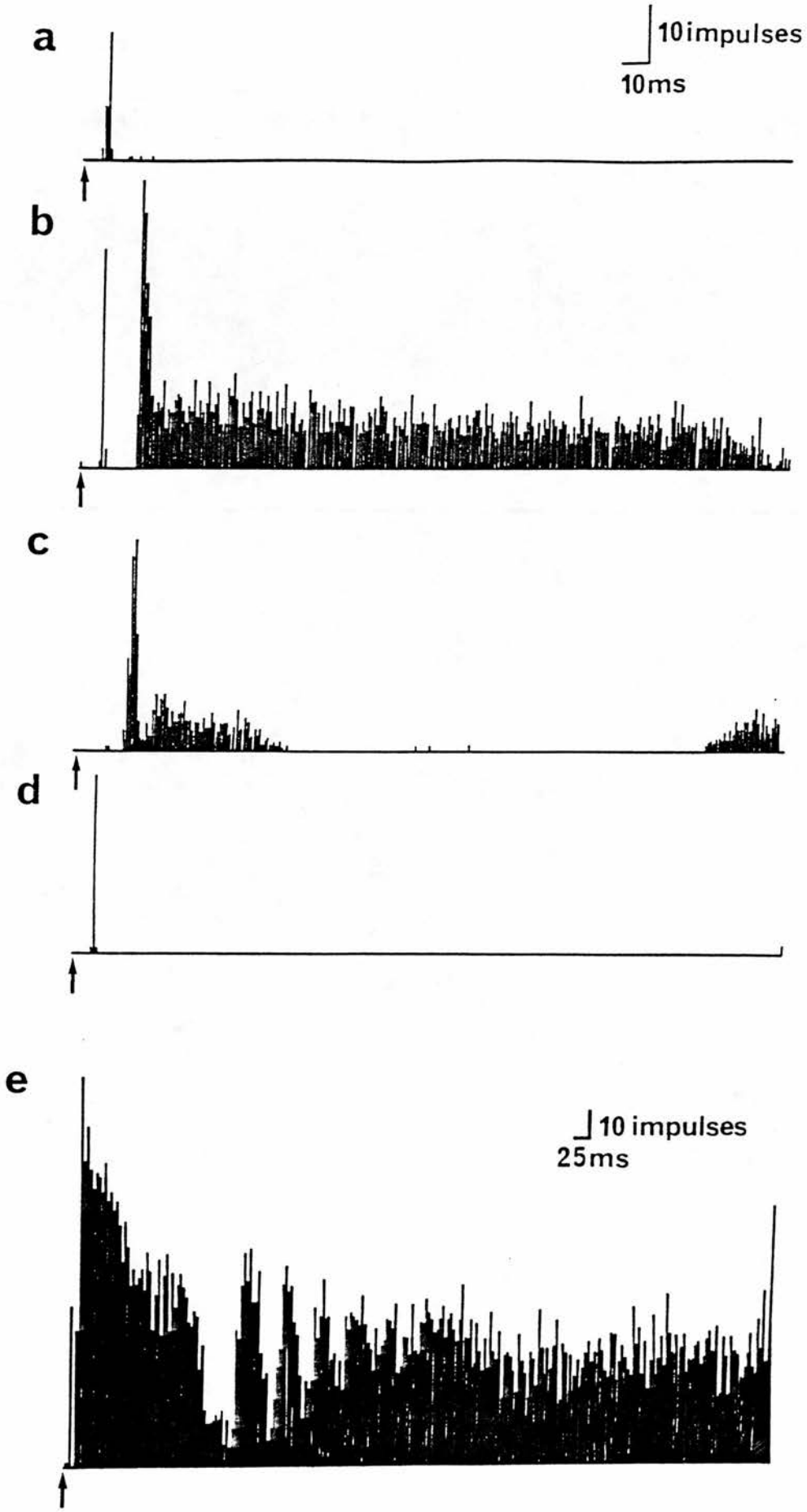
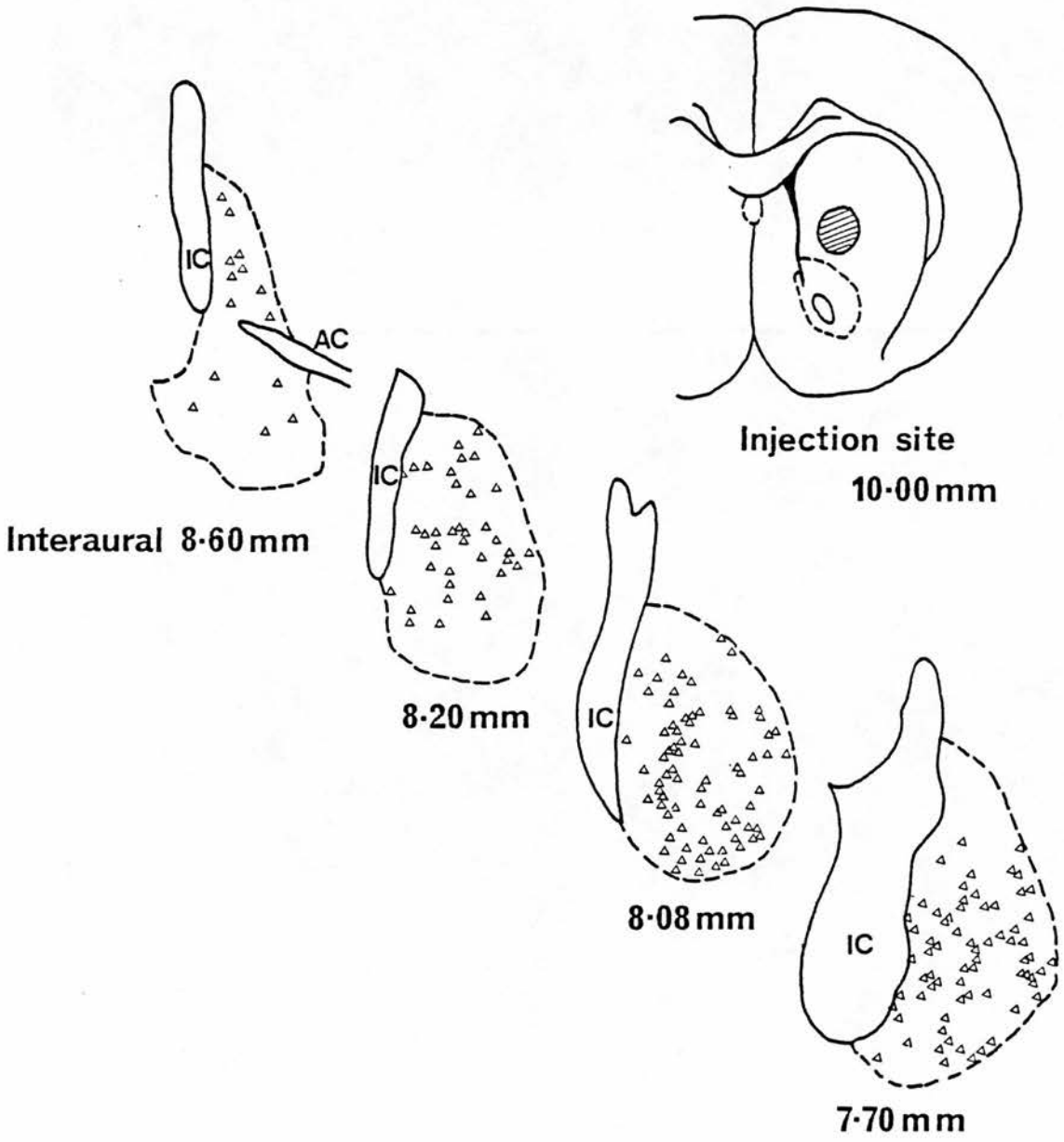


Figure 2.19 Location of injection site within the striatum, and the positions of retrogradely labelled cells in the globus pallidus, at the levels indicated Abbreviations as before, AC=anterior commissure



Chapter 3

Internal and external organisation of the neostriatum

Introduction

The neostriatum is the largest nucleus of the basal ganglia, and for a long time was thought to be a relatively homogeneous structure with no apparent macroscopic organisation of its component cells. In other parts of the brain the anatomical organisation of cells has provided clues to understanding their functional organisation. Examples of this are the cortex, the retina, and the superior colliculus, in which the anatomically distinct layers suggested a correlation with function. For a long time there was a dearth of information of this nature which might contribute to an understanding of striatal function. Studies of the cell types of the striatum have revealed many details of connectivity within and outside the striatum, but there are many pieces missing in our understanding of what the striatum does and how it does it.

After discussing the various cell types found in the striatum, I will describe the second level of organisation, the striosomal system, in terms of the many histological techniques which reveal it, including studies of extra-striatal connections. The relationship of different cell types to this anatomical division may help reveal something of their function within the striatum, and how this relates to human pathology.

Cell types

The striatal cell population is made up of a several different cell types which have been classified in a number of ways in several different species (Kemp and Powell, 1971b; Mensah and Deadwyler, 1974; Dimova *et al.*1980; Tanaka, 1980; DiFiglia *et al.*1980; Chang *et al.*1982; also see Fox *et al.*1971a; Fox *et al.*1971b; Fox and Rafols, 1971; Fox *et al.*1974; Danner and Pfister, 1979 cited in Graybiel and Ragsdale, 1983) usually first according to cell body size and then by morphological features such as dendritic spines, dendritic branching, soma shape axonal branching and length. There are also typical features of the nucleus and cytoplasm which characterise the various cell types at the electron microcope level. There are differences between species, but the similarities are such that it is possible to correlate to some extent descriptions from different authors. More recently studies have focussed upon the neurochemical identification of cell types; in some cases the morphological characteristics clearly correspond to particular neurochemical subtypes but in others they do not. This is perhaps due to distortions of anatomical features by the methods used, such as different fixation or staining techniques, or the pretreatment of the animal with colchicine.

Chang and colleagues (1982) review a number of attempts

to classify striatal cells and offer a classification based predominantly upon the size of the cell body. They describe two large cell types and five medium cell types in the rat and they correlate previous studies in other species with their classification. Pasik *et al.*(1979) describe the cells found in the primate neostriatum according to whether they have spines on their dendrites, and list two spiny cell types, and three aspiny, with a small neurogliaform cell (this cell, which had a 10um diameter cell body and profusely branching fine dendrites, is mentioned in most studies, but is almost certainly not a neuron as an axon has never been seen).

Spiny cells

Ninety-six percent of the striatal cell population as estimated in the cat (Kemp and Powell, 1971b) is made up of cells with medium sized (15-20um) cell bodies. This is the most easily identifiable type in each species studied, corresponding to Chang and co-workers' medium type 1, and Pasik and co-workers' spiny type 1. The cell body is oval; its dendrites, 4-7 in number (rat, Dimova *et al.*1980), are distinguished by the dense covering of spines starting at approximately 40um from the cell body (Kemp and Powell, 1971b; Mensah and Deadwyler, 1974; Chronister *et al.*1976; DiFiglia *et al.*1976; Pasik *et al.*1979; Dimova *et al.*1980; Wilson and Groves, 1980; Tanaka, 1980; Chang *et al.*1982; Graveland *et al.*1985b). The dendrites are approximately 200-220um

long in the rat (Dimova *et al.*1980). On the electron microscopic (EM) level, the nucleus occupies most of the cell body and is not indented (Somogyi and Smith, 1979; DiFiglia *et al.*1980; Bolam *et al.*1981b).

These cells are projection neurons (Somogyi and Smith, 1979; Wilson and Groves, 1980; Preston *et al.*1980; Bolam *et al.*1981a,b; Wilson and Phelan, 1982; Misgeld *et al.*1984; Bolam and Smith, 1990) to the globus pallidus, substantia nigra pars reticulata and pars compacta, and thalamus (see figure 1.1). The principal neurotransmitter is gamma-aminobutyric acid (GABA) (Kim *et al.*1971; Fonnum *et al.*1974) and either enkephalin or substance P is usually co-localised with it (see Chapters 1 and 2). Other neuropeptides have been demonstrated in striatal efferents which are also contained in these cells, such as neurokinin A (Lee *et al.*1986; Arai and Emson, 1986; Nagashima *et al.*1987) and neurotensin (Sugimoto and Mizuno, 1987).

These cells have extensive axon collaterals within the striatum (Preston *et al.*1980; Wilson and Groves, 1980; Bishop *et al.*1982; Penny *et al.*1988; Kawaguchi *et al.*1989) and make contact with interneurons (Bolam *et al.*1986) and with the shafts of dendritic spines of other medium spiny cells (Somogyi *et al.*1982; Bolam and Izzo, 1988a; Ingham *et al.*1991).

The medium spiny cells receive inputs from different sites onto different parts of the cells. Axons from cortical cells make contact with the heads of spines (Kitai *et al.*1976a; Kitai *et al.*1976b; Hassler *et al.*1978; Hattori *et al.*1979; Bolam *et al.*1981b; Frotscher *et al.*1981; Dube *et al.*1988; Kawaguchi *et al.*1989). Afferents from the substantia nigra pars compacta make synapses on dendritic spine necks (Kitai *et al.*1976a; Freund *et al.*1984; Kubota *et al.*1986; Hattori *et al.*1991). Axons from the cortex and the substantia nigra pars compacta were observed by Bouyer and co-workers (1984) often to make contact with the same dendritic spine. Hattori and co-workers (1991) also describe nigral afferents making contact with spine heads. These terminal boutons are not tyrosine hydroxylase-positive, but appear, somewhat paradoxically, to originate from the same cell population as the dopaminergic axons. Local interneurons synapse onto dendritic shafts and spine necks (DiFiglia and Aronin, 1982a; DiFiglia and Aronin, 1984; Takagi *et al.*1984; Wainer *et al.*1984; Bolam *et al.*1985; Phelps *et al.*1985; Izzo and Bolam, 1988). In addition, Pickel and Chan (1991) have demonstrated appositions between ChAT-positive dendrites and perikarya of medium spiny cells, suggesting a non-synaptic mechanism by which the cholinergic interneurons could modulate the activity of these cells. This hierarchy of inputs may reflect different functional effects upon medium spiny neurons.

A second category, comprising less than 1% of the striatal cell population is the medium-sized neuron which is sparsely spiny; this is Pasik and co-workers' spiny type II, which can also have a large cell body. This probably corresponds in the rat to Chang and colleagues' type III, and to Dimova and colleagues' type II; the cell body is round or elongated with a small number of dendrites with some spines, not branching extensively, but longer than for the previous type. The large type might correspond to the larger GAD-positive cells seen by Kita and Kitai (1988) in the rat.

Aspiny cells

Pasik *et al.*'s aspiny type I is characterised by varicose dendrites, and there seem to have been such cells in most of the other papers mentioned. Tanaka (1980) describes the dendrites of this type as "swirling". It corresponds in the rat with Chang and co-workers' type V and Dimova and co-workers' type III. These cells make up 1-2% of the cell population and are likely to be interneurons, being similar to the short-axon cells seen by Difiglia and Aronin in the rat (1982a). They have a highly indented nucleus, with abundant cytoplasm rich in organelles. These cells are probably one type of GABAergic interneuron (Bolam *et al.*1983a, 1985; Kita and Kitai, 1988); they are distinguished from other GABA-containing striatal cells

in the rat by staining for parvalbumin (Cowan *et al.*1990).

Cells corresponding to the large aspiny type II have been noted consistently in most species; in the rat (Mensah and Deadwyler, 1974; Dimova *et al.*1980; Chang *et al.*1982), the cat (Kemp and Powell, 1971b) and the monkey (Pasik *et al.*1979; DiFiglia *et al.*1980). It is generally accepted that these cells are cholinergic interneurons, staining positively for choline acetyltransferase (ChAT) (Bolam *et al.*1984; Mesulam *et al.*1984). The cell body is fusiform or triangular, 25-40um in diameter, with thick long varicose dendrites from both poles, and the axon emerging from the perikaryon or basal dendrite. The cytoplasm is pale with profuse aggregates of granular cisternae (Dimova *et al.*1980). Similar EM features were described in the primate by Difiglia and Carey (1986). The ChAT-positive large cells seen by Phelps *et al.* in the rat (1985) had a few dendritic spines, but otherwise are identical to the cells described by others, with an indented nucleus and organelle-rich cytoplasm.

The aspiny type III neuron in the primate has 3-4 short smooth dendrites, and a short axon with many local branches. It is unclear whether this corresponds to Chang and co-workers' medium type III depending on whether these are truly aspiny or actually possess a few spines, in which case they correspond better to medium

size spiny type II cells. They also seem to correspond to a second type of neuropeptide Y-positive cells seen in primate by Kubota *et al.* (1988), which co-localise with somatostatin and NADPH-diaphorase (Takagi *et al.*1983; Vincent and Johansson, 1983a; Vincent *et al.*1983b; DiFiglia and Aronin, 1984; Smith and Parent, 1986b; Chesselet and Graybiel, 1986; Kowall *et al.*1987), and avian pancreatic polypeptide (Kowall *et al.*1987). These cells tend to lie near the borders of striosomes (Graybiel *et al.*1981; Gerfen, 1985) (see below). Kerkerian-Le Goff and colleagues (1991) showed in the rat that all of these cells were also GAD-positive. They receive an input from dopaminergic cells of the substantia nigra (Kerkerian *et al.*1986; Kubota *et al.*1988; Vuillet *et al.*1989) from the cortex (Vuillet *et al.*1989) and from GP (Staines and Hincke, 1991).

Takagi and co-workers (1984) described two different types of aspiny cells, corresponding to the aspiny cell types I and II, and a third which did not correspond, all of which made synaptic contacts with medium spiny neurons.

Striosomes

Several descriptions have been published of cell clustering in the striatum in various species - rat, cat and mouse (Chronister *et al.*1976; Goldman-Rakic, 1982).

These were suggestive of an underlying organisation of the striatum, although there were no distinct structures described. Mensah (1980) described cell clusters in the rat, each group of medium sized cells being associated with a large neuron, and aligned either parallel or a right angles to internal capsule fibre bundles.

A more defined neurochemical heterogeneity was revealed within the striatum by Graybiel and Ragsdale in 1978. This was initially characterised by discrete variations of acetylcholinesterase (AChE) staining demonstrated in the human, monkey and cat. These investigators described areas of weak AChE staining - striosomes - set in a matrix of stronger AChE staining. This enzyme is responsible for degrading acetylcholine by hydrolysis. Acetylcholine is used as a neurotransmitter by the large interneurons of the striatum, although the enzyme is not specific to these cells (Mesulam *et al.*1984).

This internal structure of the striatum has been demonstrated by many other neurochemical and -anatomical techniques. The divisions are visible to varying extents throughout the striatum, but tend to be most marked dorsally, laterally and rostrally, sometimes even with a reversal of the pattern ventromedially. They can be demonstrated to differing extents with various markers in most species.

Immunohistochemistry to neurotransmitters

Immunohistochemistry to enkephalin, which is amongst the various neurotransmitters utilised by the medium spiny striatal projection neurons (Cuello and Paxinos, 1978; Haber and Elde, 1982; Del Fiacco *et al.*1982; DiFiglia *et al.*1982b; Penny *et al.*1986) labels cells in the matrix. Many of cells, both within and outside striosomes can be shown, by *in situ* hybridisation studies to have the capacity of producing met-enkephalin (Gerfen and Young, 1988). Different immunohistochemical protocols have been shown to produce different labelling of the two compartments (Graybiel *et al.*1981; Chesselet and Graybiel, 1983; Graybiel and Chesselet, 1984; Izzo *et al.*1987; Besson *et al.*1990).

The distribution of immunostaining can be altered by pretreatment of the animal with colchicine, which facilitates labelling of the cell bodies by inhibiting axonal transport and causing accumulation of peptide. Following colchicine administration, an increase is seen in the number of cells which stain for enkephalin within the striosomes (Graybiel and Chesselet, 1984; Besson *et al.*1990).

Substance P, which stains the perikarya and dendrites of a predominantly distinct group of efferent neurons, labels striosomes (Graybiel *et al.*1981; Chesselet and Graybiel, 1983; Graybiel and Chesselet, 1984; Beach and McGeer, 1984; Beckstead and Kersey, 1985; Gerfen, 1985; Izzo *et al.*1987; Beckstead, 1987; Bolam *et al.*1985;

Besson *et al.*1990). In an *in situ* hybridisation study, Gerfen and Young (1988) found small differences between cells in patches or matrix of the rat (61% and 54% respectively), although the trend was the same as in other studies (Penny *et al.*1986; 55% and 30% respectively). Colchicine did not seem to affect staining for substance P (Penny *et al.*1986; Besson *et al.*1990).

Dynorphin co-localises with substance P in colchicine-treated cats (Besson *et al.*1990).

When colchicine is not used, only 17% of all cells demonstrate staining for both enkephalin and substance P (Penny *et al.*1986). With the injection of colchicine, this number increases to 76% (Besson *et al.*1990). As the striatal projection neurons are in general segregated in terms of their projection sites, to LGP or MGP, and the neurotransmitters to these sites are distinct (Haber and Elde, 1982), it seems unlikely that these two peptides are normally co-expressed *in vivo*.

Immunohistochemistry for somatostatin, which is believed to be present in terminals of the striatal interneurons, labels the matrix (Graybiel *et al.*1981; Gerfen, 1985).

Labelling of enzymes

Enzymes involved in the synthesis or breakdown of

neurotransmitters can also demonstrate this patchy pattern of labelling. Antibodies to cholineacetyltransferase, which is a synthetic enzyme of acetylcholine shows the same pattern as AChE staining (Graybiel *et al.*1986). Staining for butyrylcholinesterase (BChE) (Graybiel and Ragsdale, 1978; Langer and Graybiel, 1989) using a technique modified from that for acetylcholinesterase, in the primate, produces a pattern complimentary to that of AChE, staining the striosomes. Glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, which is contained in both striatal efferents and interneurons, labels the neuropil of striosomes although GABA is a neurotransmitter of the majority of medium spiny cells throughout the striatum. Tyrosine hydroxylase from the midbrain dopaminergic system labels the matrix (Graybiel *et al.*1987). Endopeptidase-24.11 (enkephalinase), which is located in neuronal membranes in the vicinity of enkephalin receptors (De La Baume *et al.*1981) labels striosomes in pig brain (Barnes *et al.*1988).

Receptors

Autoradiography using radioactive ligands for various receptor sites, both of transmitters released from afferent axons, and from intrinsic neurotransmitters, differentially bind to striosomes and matrix. Dopamine D1 receptors are strongly localised to striosomes in the young animal, the difference becoming less marked with

age, although in some studies they still are seen to mark striosomes in the adults of various species (Richfield *et al.*1987; Beckstead *et al.*1988; Besson *et al.*1988; Murrin and Zeng, 1989) and D2 receptors in matrix (Joyce *et al.*1986; Beckstead *et al.*1988; Lowenstein *et al.*1990). Opiate receptors (Herkenham and Pert, 1981) and cholinergic muscarinic M1 binding sites are localised in striosomes (Nastuk and Graybiel, 1985; Nastuk and Graybiel, 1989; Lowenstein *et al.*1990) whereas M2 receptors are homogeneously distributed thoughout the striatum.

Other substances

Other substances whose functions in the striatum are not yet fully characterised also localise to striosomes or matrix. Some of these are putative neurotransmitters such as neurotensin (striosomes) (Goedert *et al.*1983) and dynorphin B. The latter co-localises to many of the striosomal cells which contain substance P (Chesselet and Graybiel, 1983; Graybiel and Chesselet, 1984; Besson *et al.*1990). Some substances which are known to mark interneurons such as neuropeptide Y, NADPH-diaphorase and avian pancreatic polypeptide are located in the matrix, particularly along borders with striosomes (Sandell *et al.*1986; Kowall *et al.*1987).

Calcium binding protein is used as a marker for matrix (Gerfen *et al.*1985).

Cholecystokinin (CCK) binding sites in the primate (Kritzer *et al.*1990) correlate to some extent with matrix, although CCK-poor areas do not consistently overlap with AChE-defined striosomes.
Cytochrome c oxidase localises to matrix (Augood *et al.*1989).

Connections

The internal striosome/matrix organisation of the striatum is highly significant in terms of extra- as well as intrastriatal function, i.e. in terms of its relationship to other structures.

Input to matrix

The major input to the neostriatum in terms of motor function is from the cortex. A somatotopic patchy input, predominantly to the putamen, was observed long before striosomes were described, from the primate motor cortex (Kunzle, 1975) and sensory cortex (Kunzle, 1977). Similar afferents were seen from the frontal eye field (Kunzle and Akert, 1977), from the temporal cortex (Van Hoesen *et al.*1981), and from the auditory cortex (Reale and Imig, 1983). These projections were demonstrated by Jones and co-workers (Jones *et al.* 1977; Wise and Jones, 1977) to be from pyramidal cells of layer V. Yeterian and Van Hoesen (1978) describe the projections to the

same areas of the caudate nucleus to be from cortical areas with reciprocal connections.

There is a strong correlation between afferents from sensorimotor cortex and matrix; areas receiving a strong input from frontal cortex correspond to a large extent with AChE-rich matrix (Ragsdale and Graybiel, 1981). Donoghue and Herkenham (1986) describe in the rat specific areas of motor and sensory cortex projecting to various regions of matrix. The sensory cortex in cat projects to the dorsolateral corner of the matrix (Malach and Graybiel, 1986). Graybiel and co-workers (1991) describe the clustering of terminals of afferent fibres from primate somatosensory cortex, which they term "matrisomes".

Lateral divisions of the thalamus (Ragsdale and Graybiel, 1991; Sadikot *et al.*1990) - anterior intralaminar nuclei, VA, VL and VM nuclei, CM-pf, project to the matrix. These afferents also produce a patchy pattern of labelling, maybe corresponding to "matrisomes".

Some areas which are considered part of the limbic system have been shown to project to the matrix, such as the mesencephalic dopaminergic cells of areas A8 and A10 (VTA) (Moon Edley and Herkenham, 1984; Jimenez-Castellanos and Graybiel, 1987; Gerfen *et al.*1987;

Langer and Graybiel, 1989). Gerfen and co-workers (1987) also identify a non-dopaminergic component of this projection. The pattern of the dorsal striatum is often strikingly reversed in the ventral striatum, which is more associated with limbic functions; Kita and Kitai (1990) produced anterograde labelling of the matrix in the rostroventral striatum from the basolateral amygdala.

Output from matrix

Both the major efferent pathways of the striatum are made up of neurons of the matrix. In the rat (Kawaguchi *et al.*1990) cells fell into three groups, with axon collaterals to GP, EP, and SNr, or to GP and SN, or to GP alone. The collateralisation to GP and EP is surprising because the neurotransmitter content of each pathway is different (see Chapter 2). In higher species these projections are separate, demonstrated in the cat (Graybiel *et al.*1979; Beckstead and Cruz, 1986) and in the primate (Parent *et al.*1989b; Gimenez-Amaya and Graybiel, 1990). They are from cells located in the matrix (Graybiel *et al.*1979; Jimenez-Castellanos and Graybiel, 1989; Gimenez-Amaya and Graybiel, 1990; Selemon and Goldman-Rakic, 1990). The cells comprising these pathways appear to be clustered to form "matrisomes" in a manner similar to afferent fibres (Jimenez-Castellanos and Graybiel, 1989; Selemon and Goldman-Rakic, 1990; Graybiel *et al.*1991; Gimenez-Amaya and Graybiel, 1991).

In the rat (Loopuijt and van der Kooy, 1985) there are axon collaterals to GP and VTA/SN, although the experiments of Gerfen and Young (1988) indirectly suggest otherwise. Again, in higher species the striatopallidal projection is distinct from the striatonigral projection (Feger and Crossman, 1984; Parent *et al.*1984).

The striatal efferents to the substantia nigra (Jimenez-Castellanos and Graybiel, 1989) appeared similarly clustered in the matrix. Gerfen (1984, 1985) identifies striatonigral matrix cells from injections of retrograde tracer into the substantia nigra pars reticulata of the rat.

Input to striosomes

Olson and co-workers (1972) saw histofluorescent dopamine "islands" in the neonatal rat striatum; these correspond to the projection from cells of substantia nigra pars compacta densocellular zone and pars lateralis to striosomes (Moon Edley and Herkenham, 1984; Jimenez-Castellanos and Graybiel, 1987; Gerfen *et al.*1987; Langer and Graybiel, 1989). Beckstead (1985) labelled what were probably striosomes anterogradely from cat substantia nigra, with the pattern reversing ventrally so that the labelling was more homogeneous with lacunae which were filled with terminals from the

thalamus CM-pf. Kalil (1978) produced patches of anterograde labelling in the putamen from the CM-pf in the rhesus monkey.

Paraventricular and rhomboid thalamic nuclei project to striosomes (Ragsdale and Graybiel, 1991).

The striosomes receive input from areas related to the limbic system, e.g. the prelimbic cortex (Gerfen, 1984; Donoghue and Herkenham, 1986) and the basolateral amygdala (Ragsdale and Graybiel, 1988) (but see Kita and Kitai, 1990).

Ragsdale and Graybiel (1990) have demonstrated cortical terminals labelling striosomes dorsally, from prefrontal, insular and temporal cortex in the cat. The labelling pattern reverses to label matrix ventrally, at levels which vary dorsoventrally with the area of cortex. This is suggestive of some level of cortical organisation which has not yet been elucidated.

Output from striosomes

The medium spiny cells which form the striatonigral projection to the pars compacta are mainly located inside striosomes (Graybiel and Chesselet, 1984; Gerfen, 1985; Izzo *et al.*1987; Jimenez-Castellanos and Graybiel, 1989).

Cells and Borders

The differences in histochemical staining which describe the striosome/matrix structure are caused by variations in the histochemical nature of the striatal neuropil. Thus it would be expected that labelling the tissue for different neurotransmitters would demonstrate dendrites, axons and cell bodies which define striosome/matrix boundaries. This can be discussed in relation to interneurons and to projection neurons.

Interneurons

Striosomes were initially revealed by staining for acetylcholinesterase, which in the striatum corresponds to a high degree with the immunohistochemistry for cholineacetyltransferase. This also labels the extrastriosomal matrix (Graybiel *et al.*1986) and stains specifically the neuropil of the large cholinergic interneurons. Apparently paradoxically the cholinergic cell bodies themselves are not confined to either compartment, but tend to be clustered in and around striosomes (Martone *et al.*1988, 1991). The dendrites and axons of cholinergic cells studied in isolation do not appear to follow the configuration of striosomal borders, and are often seen to cross them (Penny *et al.*1988; Wilson *et al.*1990).

Borders are also crossed by the other types of interneurons. The medium size aspiny GABAergic cell,

can be situated in patch or matrix, with axons and dendrites crossing the borders (Cowan *et al.*1990). Gerfen (1984), in the rat, similarly found somatostatin-immunoreactive interneurons to be situated in either compartment, although these cells had their processes predominantly in the matrix.

Projection neurons

Immunohistochemistry to neurotransmitters, such as enkephalin and substance P which are used by the projection neurons, also demarcates striosomes. In the rat (Penny *et al.*1988; Kawaguchi *et al.*1989), labelling intracellularly with HRP, the dendrites of these cells avoided crossing borders. Sometimes sharp bends in the distal tips of the dendrites - "recurved ends" - were seen when dendrites were near a border. This was similarly observed in the rat by Gerfen (1985). However, in the cat and ferret, using a combination of Golgi and immunohistochemical techniques, Bolam *et al.* (1988) described various different patterns of the dendrites of medium spiny cells. They observed that whilst some cells were strongly oriented to avoid crossing borders, a significant number of others did not respect striosome/matrix boundaries. A third category was cells situated in the matrix distant from borders which were apparently not influenced by them.

Cells with dendrites arborising primarily within one

compartment receive a different type of information than cells in the other compartment (figure 3.1); they have inputs from different extrastriatal sources, sometimes with different neurotransmitters. Although they appear identical morphologically and physiologically (Kawaguchi *et al.*1989) they have different types or concentrations of receptors.

If the medium spiny cells are not strictly segregated in terms of their compartment, then inputs to one compartment can directly influence the output cells of the other, and there could be extensive overlap and cross-communication between the striosomes and matrix systems.

Spatial Relationships

There has been no work to date which has suggested a particular orientation of striatal neurons of any type. This is perhaps surprising given the usefulness of this type of information in forming hypotheses about neuronal function, for example in the cortex and retina. Percheron and colleagues' work upon the three-dimensional orientation of pallidal cells in the primate (Francois *et al.*1984; Percheron *et al.*1984; Yelnik *et al.*1984), along with estimates of dendritic field size, have led them to build a model of basal ganglia function in which incoming striatal axons traverse the large dendritic fields of large pallidal neurons, which are

optimally oriented with their long axes parallel to the external surface of GP, so as to be exposed to a large number of incoming axons.

In their work they represent striatal dendritic fields as spherical; the dendritic fields of medium spiny striatal cells are far smaller (diameter up to 400um) than those of pallidal cells (long diameter up to 1500um), so it is unlikely that a similar convergence of information would take place. However, there is at present no information pertaining to this aspect of the cells which make up 96% of the striatal cell population. Although many details of intercellular relationships have been described, remarkably little is known about their function. The relationship of these cells to striosomes, the study of which has given many clues about striatal function, but no clear answers, may contribute towards a further understanding of basal ganglia function.

Summary

The major effect of basal ganglia dysfunction in humans is one of loss of control over motor function. In terms of motor control, the input from the sensorimotor cortex is predominantly to the extrastriosomal matrix, particularly dorsolaterally where the striosomes,

defined as AChE-poor zones, are most clearly distinguished. The output of this area, from the medium spiny cells, is to the main descending motor pathways, via the globus pallidus internal segment, and the substantia nigra pars reticulata.

The striosomal system relates more to the limbic system, receiving input from the limbic-related cortical areas, however this division of function is not as clear as it once seemed, especially in the ventromedial striatum, which is closely associated with the nucleus accumbens.

Thus it appears that the "motor" and "limbic" systems may be less of an anatomical than a functional distinction, and that the two may have extensive influence upon each other.

Studies of striatal cells in the ferret

Introduction

The initial proposal was for a study, anatomical and subsequently electrophysiological, of the cholinergic interneurons of the striatum (McGeer *et al.*1971a; Lehmann and Langer, 1983; Bolam *et al.*1984). As described in the previous section, from anatomical studies, these cells would appear to play an important role in communicating between striosomes and matrix. There are also clinical indications of their

significance. Some of the symptoms of Parkinson's disease, such as tremor and rigidity, can be relieved by anticholinergic therapy, which suggests that the loss of the dopamine of the nigrostriatal tract results in an effective overactivity of cholinergic cells. This also suggests that the two neurotransmitters affect separate neuronal paths, which is supported by the fact that different clinical syndromes can be seen with features of different deficiencies.

Knowing more about the cholinergic interneurons of the striatum, which are in a position to strongly influence the cortical inputs to the striatum, and the role they play in integrating striatal function, would give us important information as to their behaviour in Parkinson's disease.

I aimed to study the relationships of the cholinergic interneurons to striosomes, by filling them with a dye, and looking at their dendrites with respect to histochemically-labelled striosomes. These neurons make up a very small percentage of the striatal cell population (1-2%) (Kemp and Powell, 1971b) and are thus difficult to characterise using the technique of intracellular recording and filling which labels a relatively random selection of the cell population (Penny *et al.*1988; Wilson *et al.*1990). A method for identifying the cholinergic cells in a slice preparation

would make it possible to see them down the microscope, eliminating the need to hit them by chance. They could then be targetted using a micropipette filled with dye, and identified cells could be filled, enabling them to be characterised anatomically. The tissue would subsequently be processed so that striosomes were visible, and the relationships of the dye-filled cells to the striosomes could be described.

The technique of intracellular filling has been widely used, usually with horseradish peroxidase, in combination with intracellular recording in living tissue. Cells which have been described neurophysiologically can be identified and described anatomically. Various workers have carried out this type of work in the rat neostriatum (Kitai *et al.*1976a; Preston *et al.*1980; Wilson and Groves, 1980; Wilson and Groves, 1981; Bishop *et al.*1982; Penny *et al.*1988; Kawaguchi *et al.*1989). Much detailed physiological information about each cell can be obtained, but the yield is relatively low in terms of numbers of cells, and the vast majority of cells labelled were of the medium sized, densely spiny type.

It was necessary to find a way of identifying the large cholinergic cells. A technique that is often used to label specific neuronal subpopulations is retrograde labelling by injecting a tracer into the area of termination of the cells' axons (Grofova, 1975;

Bentivoglio *et al.*1979). The label is picked up by the axon terminal uptake mechanism, and is retrogradely transported by axonal transport to the cell body. Cells labelled with the tracer would then be identifiable in the slice and could be selected for more specific study (Misgeld *et al.*1984; Katz *et al.*1984; Katz, 1987; Asanuma, 1988; Wouterlood *et al.*1990). However, because the cholinergic cells are interneurons it is not possible to label them by this method. There is some evidence that interneurons can be labelled from intrastriatal injections (Chesselet and Graybiel, unpublished) but my initial experiments, with a variety of tracers, including rhodamine-labelled beads, failed to reveal any significantly specific labelling of large cholinergic interneurons, so other ways were tried of identifying these cells.

I decided to try to identify them initially in a fixed slice; optimally the method of choice would be transferable to a living slice set-up, but it was decided to try and get a method to work in a relatively simple situation first, without the complications of electrophysiology. Once identified, labelled cells could be targetted under visual control, by a micropipette filled with fluorescent dye, such as lucifer yellow-CH (Aldrich/Sigma) (Stewart, 1978), and filled so that all anatomical details were visible (although not usually axons). In fixed tissue cells can

be filled with dyes and studied anatomically (Tauchi and Masland, 1985; Einstein and Fitzpatrick, 1987; Asanuma, 1988; Buhl and Lubke, 1989). Although it is not possible to collect electrophysiological information about the filled cells, as is possible in a living slice, the yield in terms of numbers of cells is much greater. Despite the apparent simplicity of this technique, there were many technical problems to be overcome before cells could be filled consistently and satisfactorily.

The ferret was chosen for these experiments, as it is developmentally higher than the rat, with a clearly-developed striosomal system, which is not broken up by fibre bundles.

A satisfactory method of fixation was achieved following trials many different techniques. Although similar work has been done in the retina (Tauchi and Masland, 1985) and in other tissue preparations (Einstein and Fitzpatrick, 1987; Asanuma, 1988; Buhl and Lubke, 1989) the optimal preparation of slices of ferret brain for cell filling turned out to be quite different. Subtle variations in the amounts of the different constituent fixatives resulted in great differences in the quality of the tissue. Eventually I arrived at a perfusion recipe and protocol which resulted good preservation of cellular structure.

Methods

Slice preparation

Tissue slices were obtained from adult ferrets. Each animal was given a lethal intraperitoneal dose of sodium pentobarbital (Nembutal) and perfused through the heart with 0.9% sodium chloride (room temperature, approximately 300ml) followed by a solution of 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1M dibasic phosphate buffer containing 0.9% sodium chloride (room temperature, approximately 300ml). The brain was rapidly blocked *in situ*, and the block containing the striata was removed, chilled for up to one minute in 0.1M phosphate buffer, and bisected along the midline. Trimmed blocks containing the caudate nucleus and putamen were sliced coronally, sagittally or horizontally at 400um on a vibratome. Slices were collected in chilled 0.1M phosphate buffer, floated onto small squares of filter paper to keep them flat, and stored wet at $4^{o}C$ until use. To keep the slices damp and to inhibit bacterial growth they were stored in a petri dish lined with filter paper soaked in 0.1M phosphate buffer containing 0.1% sodium azide.

Preliminary experiments

With the initial aim of studying the large interneurons, it was necessary to develop a way of identifying and filling cells in the slice which was compatible with subsequent labelling of striosomes. A number of

different techniques were tried in order to identify these cells; for example, staining the whole slice for acetylcholinesterase (Geneser-Jensen and Blackstad, 1971; Graybiel and Ragsdale, 1978; Satoh *et al.*1983), after injecting the animal with diisopropylphosphofluoridate (DFP) (Butcher *et al.*1975; Somogyi and Chubb, 1976). This method is known to produce labelling of the cholinergic cell bodies in the striatum (Levey *et al.*1983; Mesulam *et al.*1984). This was not satisfactory because the tissue was too thick for labelled cells to be seen with transmitted light.

A number of fluorescent cell stains were tried without success; 4,6-diamidino-2-phenylindole (DAPI) (Masland *et al.*1984; Sandell and Masland, 1986), ethidium bromide (Schmued *et al.*1982), acridine orange (Mai *et al.*1984), 1,1',dioctadecyl-3,3,3',3'tetramethylindocarbocyanine perchlorate (di-I) and 3,3'-dioctadecyloxacarbocyanine perchlorate (di-O) (Honig and Hume, 1986; Thanos and Bonhoeffer, 1987; Godement *et al.*1987). Some of these were not very satisfactory because they stained only the cell nuclei and thus different cell types were not readily distinguishable. A method of staining all cells with a fluorescent Nissl-like cytoplasmic stain called meta-phenylene diamine (m-phd) (Quinn and Weber, 1988; Quinn, 1989) initially appeared feasible (Walker *et al.*1988); large, putatively cholinergic cells were readily distinguishable from other cell types but this

method was later abandoned because the meta-phenylene diamine stain obscured the lucifer yellow-filled dendrites in the final section. It also appeared that it might be very difficult to fill these large cells with enough dye to enable their processes to be seen, due to their large intracellular volume (figure 3.2). Eventually cells were targetted at random in a fixed striatal slice, omitting the metaphenylene diamine. The large number of cells filled by this method meant that a variety of cell types were seen, including less frequent types such as the large interneurons.

Cell filling

Each slice, supported on a piece of filter paper, was placed in a small bath on the stage of a microscope equipped with an epifluorescence source and a filter for an excitation wavelength of 470nm. The slice was kept moist, but not floating, with frequent applications of 0.1M phosphate buffer. A thin layer of buffer maintained electrical contact between the pipette and the earth connection. Cells within the striatum were impaled at random under visual control with a micropipette pulled from capillary glass containing a filament (A-M Systems Inc., Everett, WA) and filled with 8% lucifer yellow (LY) (Aldrich/Sigma) in distilled water (resistance approximately 50 MOhms). Cells were filled with the dye using negative current pulses of a few nanoamps, seven seconds on/seven seconds off, until the distal dendrites were bright up to their tips, and

could be seen to stop cleanly. Filling time varied with the size of the cell, but was usually complete within a few minutes. Sites for attempting fills were chosen approximately 300um apart so that dendritic fields would not overlap and cells could be distinguished at the reconstruction stage.

It was necessary to find a good way of detecting the striosomes in the ferret. Staining for acetylcholinesterase (Geneser-Jensen and Blackstad, 1971; Graybiel and Ragsdale, 1978) was initially tried, but this did not produce good results in the ferret. Sectioning the slices at 50-60um, and staining them for butyrylcholinesterase (Graybiel and Ragsdale, 1978) produced clear striosomal labelling and was compatible with preservation and visibility of the dye-filled cells (figures 3.3, 3.4(a), 3.14(c), 3.17(b)).

Striosomal labelling

After a number of cells had been filled in a slice, it was postfixed for 1/2-4 hours in 4% paraformaldehyde in 0.1M phosphate buffer, and sunk in 30% sucrose. The slice was then sectioned at 50um intervals on a freezing microtome.

After a wash in buffer/saline the slices were incubated in butyrylcholinesterase reagent. This is modified from the Jensen-Blackstad copper thiocholine

acetylcholinesterase stain by sustitution of butyrylthiocholine as the substrate at triple concentration, and the use of an acetylcholinesterase inhibitor (Geneser-Jensen and Blackstad, 1971; Graybiel and Ragsdale, 1978).

For 100ml, dissolve in 100ml distilled water:

1,5-bis(4-allyldimethylammoniumphenyl)-pentan-3-one dibromide (Burroughs-Wellcome/Sigma)	0.069g
butyrylthiocholine iodide	0.057g
glycine	0.075g
copper sulphate	0.05g
sodium acetate	0.41g
drops of glacial acetic acid to pH5	

The sections were incubated until a white precipitate formed (6-8 hours). They were reacted in 10% ferricyanide in distilled water, until the white precipitate turned red-brown. The reaction was stopped in distilled water, and the sections rinsed in 0.9% saline.

Sections were then mounted out of 0.5% gelatin on 50% ethanol and air-dried; after a brief wash in distilled water to remove salts, they were defatted through increasing concentrations of ethanol to 100% and then 100% xylene and coverslipped in DPX.

In order to improve preservation of filled cells, and to

avoid the problem of photobleaching of lucifer yellow during drawing, several techniques were tried which would produce a photodense reaction product with lucifer yellow. An antibody to lucifer yellow (Taghert *et al.*1982) and the diaminobenzidine (DAB) photoconversion method (Sandell and Masland, 1987), were tried, both without success at this juncture. Eventually the problem was minimised by storing the tissue at - 70^{o}C, which also reduced the development of background fluorescence, and by drawing the cells with a minimum amount of illumination.

Recording of data

The striosomes were drawn first, on a Biocom system (Biocom Inc., Paris). The advantage of this over the Eutectics system was that a photograph of the image of the tissue could easily be taken as a permanent record. This was necessary as the BChE stain darkened under fluorescent light and the striosome borders became obscured. The image produced using a 4x objective under visible light was viewed on a video screen, and the outlines of the striosomes, along with local landmarks such as blood vessels, were drawn (figure 3.4(b),(c),(d)). Under fluorescent illumination, at higher power, the positions of lucifer yellow-filled cell bodies were also marked on this low power map in order to facilitate correlation of cell locations with

striosomes. The video image with and without the graphic overlay was photographed before drawing cells, as a permanent record.

Cells were then drawn at higher power (63x or 100x) using the Eutectics Neuron Tracing System (Capowski and Sedivec, 1981; Capowski, 1985) (figure 3.4(b)). Local landmarks such as blood vessels were drawn in wherever possible. Cut dendrites in adjacent sections were matched up with their cell bodies to make a complete dendritic arbor. The drawing of each cell (or its cut dendrites) was then superimposed upon the striosome map and the relationships of dendrites to striosomal borders could be seen (figure 3.4(b)).

Cells were drawn from a coronal slice, a horizontal slice, and a sagittal slice, although because of technical limitations orientation analysis was not performed upon the sagittal slice.

Analysis of data

In order to determine the orientation of the cell with respect to the striatum, vector diagrams were constructed of each cell (see figure 4.9 for examples). The length of dendrite in each of 24 fifteen degree segments around the cell body in the plane of the section, is repesented as a value for each segment. These values can be written as ASCII files and used to perform statistical analysis upon the cell structure.

Cells were analysed from the coronal slice and the horizontal slice; for each of these cells were classified as whether they were located near striosomes or not, to determine whether the orientation of the dendritic field was related to the proximity of a striosome/matrix border. As cells filled were located relatively near the top of the slice, one would expect dendritic trees to be less complete in this direction. In order to test if the orientation data was biassed by including incomplete cells, cells with their cell bodies located on deeper sections were examined separately.

Values were pooled for all cells to be included in each group, and the mean value and standard deviation for each 15^{o} segment was calculated. To compare octants, each three consecutive values were pooled, to get values for $0-45^{o}$, $45-90^{o}$ etc., and compared by ANOVA against the values for the other seven octants. Similarly the quadrants were compared, each against the other three. Complementary pairs of quadrants ie $0-90^{o}$ and $180-270^{o}$ v. $90-180^{o}$ and $270-360^{o}$ were compared for each group.

Results

Description of cells

Cells filled in three slices are summarised in tables 3.1, 3.2 and 3.3. Forty-six medium spiny cells were filled with lucifer yellow were drawn and are described here. Thirty-five of these had their cell bodies located outside striosomes, seven were inside striosomes, three were in border regions, and one was not possible to classify. There were two large cells and two medium aspiny cells filled.

Of the 35 medium spiny cells located in the matrix, 5 had dendrites which crossed into striosomes by at least 50um (F22023), one of these entering two striosomes (C21981) (figure 3.5). One cell, C21937, clearly had a 25um length on dendrite in a striosome, whilst another (c22002) had several 25um lengths clearly within a border.

Thirteen matrix cells were far away from striosomes and their dendrites did not reach borders (eg cell2, cell18, cell8, C21957, C21989) (figure 3.6). Some cells, such as F21987 and C21996 were strongly oriented, although not near striosomes (figure 3.6). Fifteen had tips of dendrites entering striosomes by 10-20um (eg cell12, cell1, cell16, C22014, C21911) (figures 3.7, 3.8). Some cells, for example, cell6 and cell21 had dendrites which partially outlined the border of a striosome, with the

distal ends of the dendrites within the border (figure 3.9). Cell4 sent a number of dendrites towards the nearby border, yet only the tips crossed into the striosome (figure 3.9).

Of the 7 medium spiny cells situated within striosomes, 4 had dendrites which crossed out into matrix, one (C21932) sent only one dendrite out (figure 3.10), cells F21950 (figure 3.10) and C21923 had several dendrites in the matrix. C22009 had only tips out in the primary section, but on an adjacent section containing long lengths of dendrite, there was no visible striosome (figure 3.10). Three had dendrites which appeared to stay inside a striosome (cell14) (figure 3.11). There were three cells which were located in border regions, and sent dendrites out into the matrix and into the striosome (cell3, cell10, cell7) (figure 3.11). Cell3 also had labelled a group of fine axon collaterals which went out into the matrix. An additional cell (cell20) was harder to assess, but was situated within a matrix on one section, and had dendrites in the striosome on the adjacent section (figure 3.12).

Of the large cells, one was located on a border and sent dendrites in both directions (F21929), one was located in the matrix, far from a striosome (F2191b), and the third was inside a striosome and sent dendrites out into the matrix (FW2196) (figure 3.13). These cells were

probably cholinergic interneurons.

The two medium size aspiny cells were in the matrix (FW2199, cell5) and had dendrites which went in and out of a nearby striosome (figure 3.13). These both resembled the somatostatin-containing type of interneuron.

Orientation analysis

These results are presented in figures 3.14- 3.19.

The significantly different octants were in each case separated by 90^{o}, and suggest a distinctive orientation to striatal cells, parallel to the long axis of striosomes.

Problems

Sample bias

Most of the cells filled were medium-size densely spiny neurons (Kemp and Powell, 1971b; DiFiglia *et al.*1980; Dimova *et al.*1980; Chang *et al.*1982; Bishop *et al.*1982). As there was no means of targetting specific cells within the striatum, the sample would ideally be a random one of all the cell types in the striatum. The dimensions of the tip of the micropipette can determine the size and shape of cell which is filled, because of the radius of curvature. Also the procedure involved

making a judgement, as each cell was impaled by the pipette, as to whether the cell was going to fill well, as indicated by how well the dye spread through the cell body and into the proximal dendrites. If a cell appeared damaged, for example, if it was close to the top of the slice it would be abandoned. Thus any cell type which, for whatever reason, did not fill so well or rapidly by these criteria, would be selected against. Small cells or cells with few proximal dendrites might be more readily abandoned than larger cell types. However a number of the small neurogliaform cells (Fox *et al.*1974) were easily filled, as a very small amount of dye was required to fill them (see figure 4.4(b)).

Cell filling

Correct fixation was critical for cell filling. In tissue which was inadequately fixed the cell membranes were leaky and easily torn by the micropipette. When filled, "baggy", or irregularly varicose dendrites were seen, which did not retain the dye. In slices which were overfixed, the cell membranes seemed tougher and harder to penetrate with the micropipette. The dye did not flow readily to the ends of the dendrites.

Cells were filled for as long as possible, or until they were uniformly bright to the ends of the dendrites. It was clear when the dendrites were filled to their ends, as there was a sharp end to the process, rather than a

gradual tapering off of the brightness of the dye. Axons were seen only occasionally, and tended to fill better in preparations which were somewhat underfixed for dendritic filling. They did not fill as well as dendrites in the usual protocol, and if they were filled they were harder to see.

Large cells, which were probably cholinergic interneurons, were seen fairly frequently, but were very difficult to fill further than the proximal dendrites. The volume of the cell body and proximal dendrites was so large that it was very difficult to get enough lucifer yellow into them. Either it was not possible to keep the pipette in the cell for long enough, because of drift of the platform or the slice, or else the pipette became blocked. However it was usually possible to get some information from these cells in terms of the directions in which their dendrites pointed. This was also possible with a few other cells, where the ends of the dendrites were not adequately filled, but it was still possible to get information about direction of dendrites and relationship to striosomal borders.

Plane of section

Cells were analyzed from two slices, one cut coronally and one cut horizontally, in order to get information about the three-dimensional behaviour of dendrites. It is possible to get information from the neuron tracing system about dendrite configurations in the z axis, i.e.

perpendicular to the plane of section, but this is far less accurate that measurements made in the x and y directions because the judgements made by the observer as to the path of the dendrite are based upon at what level of focus the dendrite is most clearly seen, rather than superposing a cursor over the dendrite in the x and y dimensions. In fact, when a cell's dendritic tree was matched up with its cut branches in subsequent sections, the results were usually good, indicating that a reasonable degree of accuracy was achieved in the z direction.

The border question

The butyrylcholinesterase stain often resulted in clear borders between striosomes and matrix, however, on other occasions there was a gradual gradient of stain from striosome to matrix. If the exact location of the border was unclear, the line was drawn at the outermost limit of where it could be. Sometimes two contours indicated a gradient of stain. Usually the length of the dendrites was such that there was no doubt that they crossed from one compartment to another. In some cases the tips of dendrites entered the border region; this was not counted as crossing.

In some cases it was unclear whether the dendrites would have reached a border if the cell was better filled, or whether they avoided it. It was difficult to say that

dendrites of a particular cell avoided crossing a border, because a dendrite might have been missed. Sometimes in the final section it was not clear if a dendritic ending was natural or due to an incomplete fill. Thus it is easier to describe the presence of a particular type of dendritic pattern, rather than its absence.

It could be possible that a dendrite appeared to be within a striosome when it was not, because the striosome finished part way through the section. If this was a possibility, the subsequent section was examined to check for the continuation of the striosome.

Inaccuracies in the data

It can be argued that because we were working at the limits of the systems considerable inaccuracies could arise in the positioning of the cell bodies. Metrical inaccuracies in both the Eutectics cell-drawing system and the Biocom drawing system would combine with uncertainties about the exact location of the striosome border, due to fuzziness of the BChE stain. There are without doubt cases where the cell body has been displaced by some small but unknown amount, but by comparing the reconstruction with the original material at the stage of deciding whether dendrites do or do not enter a striosome, it has only been said that they do, if they do so unequivocally. Where the tips of dendrites enter a border region, this is described as such, and no

assumptions are made.

Although exposure of the filled cell to fluorescent light for the length of time it took to draw the cell resulted in fading of the dye and burning of the surrounding tissue, it was still possible to redraw a cell, if necessary, with minimal loss of detail. Working at the limits of both graphics systems resulted in small positional errors, but these were not large enough to significantly alter the information from each cell.

Discussion

We have collected data from a number of striatal cells filled intracellularly with lucifer yellow. The current results support those of Bolam and co-workers (1988b) in the cat and ferret, in that some dendrites of medium spiny cells cross out of the compartment in which their cell body is situated. This is seen in cells in both matrix and striosomes, and also in cells which are located on the border. The dendritic patterns of many cells are influenced by striosome borders, but with this stain the tips of dendrites were often seen to cross borders.

This conclusion is in contrast to the work carried out in the rat, by Penny and co-workers (1988) and Kawaguchi

and co-workers (1989). Cells in the striatum were filled with horseradish peroxidase following electrophysiological characterisation, and striosomes were labelled in the same sections using immunohistochemistry for calcium binding protein. None of the medium spiny cells thus identified had dendrites which crossed from one compartment into another; all stayed strictly within the compartment in which their cell body was located.

The different results seen here may be due to a species difference between rat and ferret, or due to a difference in sampling bias between the methods. Their significance is discussed together with the results of Chapter 4.

Table 3.1 Cells from coronal section FW21/9: location of cell body and dendrites with respect to BChE-dense striosomes

Cell#	type	cell body	dendrites
FW2199	m asp	out	in/out
C21921	m sp	out	in/out
F21923	m sp	out	in/out
F21928	large	border	in/out
F21941	m sp	out	in
F21950	m sp	in	in/out
F21966	m sp	out	in
C21970	m sp	out	out (not near striosome)
C21975	m sp	out	out
C21989	m sp	out	out (not near)
C21992	m sp	out	avoid
F2191B	large	out	out
F2191C	m sp	out	out (not near)
C21937	m sp	out	one dendrite in
C21911	m sp	out	tips only enter
C21932	m sp	in	one dendrite out
CW2191	m sp	out	out (not near)
FW2196	large	in	out
FW2194	m sp	in	in
C21957	m sp	out	stop short of border
C21996	m sp	out	stop short of border
C21981	m sp	out	enter 2 striosomes
F21987	m sp	out	out (not near)

m sp=medium spiny
m asp=medium aspiny

Table 3.2 Cells from horizontal slice FW23/8: location of cell body and dendrites with respect to BChE-dense striosomes

Cell#	type	cell body	dendrites
c1	m sp	out	tips in
c2	m sp	out	tips in
c3	m sp	border	in/out
c4	m sp	out	tips in
c5	m asp	out	in
c6	m sp	out	tips in
c7	m sp	in	in/out
c8	m sp	out	out (not near)
c9	m sp	out	out
c10	m sp	border	in/out
c11	m sp	out	out (not near)
c12	m sp	out	tips in
c13	m sp	out	tips in
c14	m sp	in	stay in
c15	m sp	out	tips in
c16	m sp	out	tips in
c17	m sp	out	out
c18	m sp	out	out (not near)
c19	m sp	in	in
c20	m sp	in	in/out
c21	m sp	out	tips in
c22	m sp	unclear	in

Table 3.3 Cells from sagittal slice FW21/22

Cell#	type	cell body	dendrites
c22002	m sp	out	tips in
c22009	m sp	in	in/out
c22014	m sp	out	tips in
c22018	m sp	out	out 1 tip in
f22022	m sp	out	1 tip in
f22023	m sp	out	out/in

Figure 3.1 Striatal efferents are only influenced by cortical input to the same compartment; integration of information between compartments is carried out by interneurons

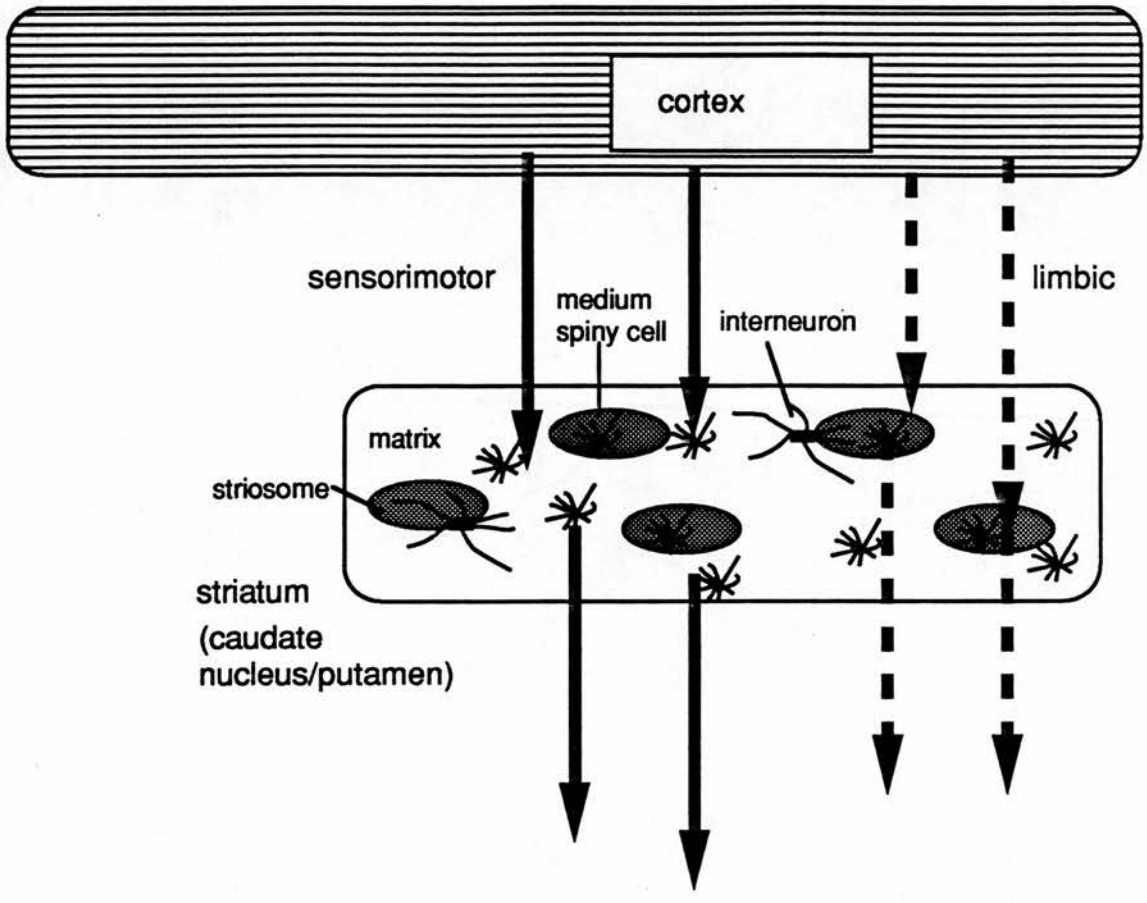


Figure 3.2 Large, presumptively cholinergic, cells filled with lucifer yellow in the fixed striatal slice; the cell bodies are approximately 40um long, with thick smooth dendrites.

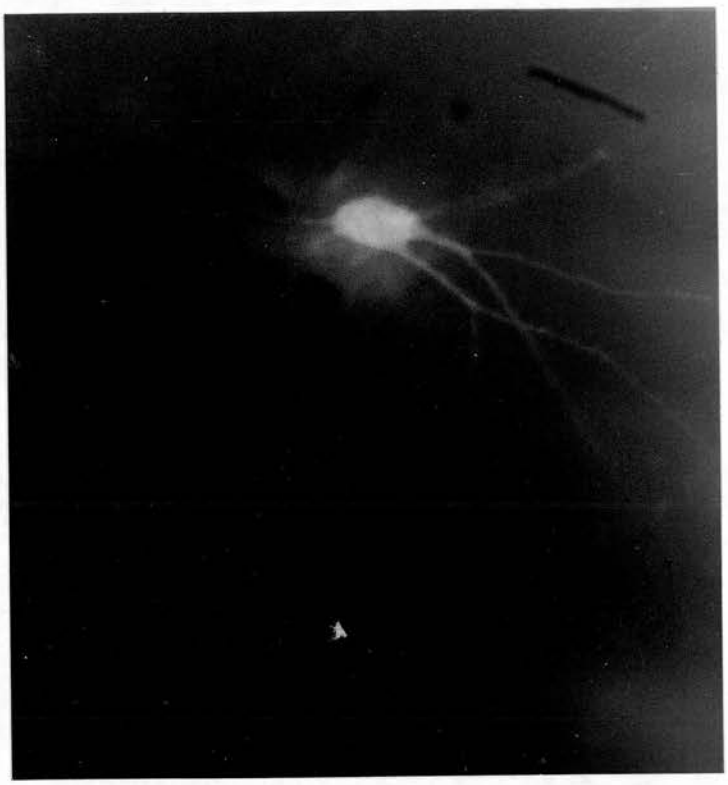


Figure 3.3 Lucifer yellow-filled medium spiny cell after processing and mounting; the cell body is approximately 15um in diameter, with branching spine-covered dendrites.

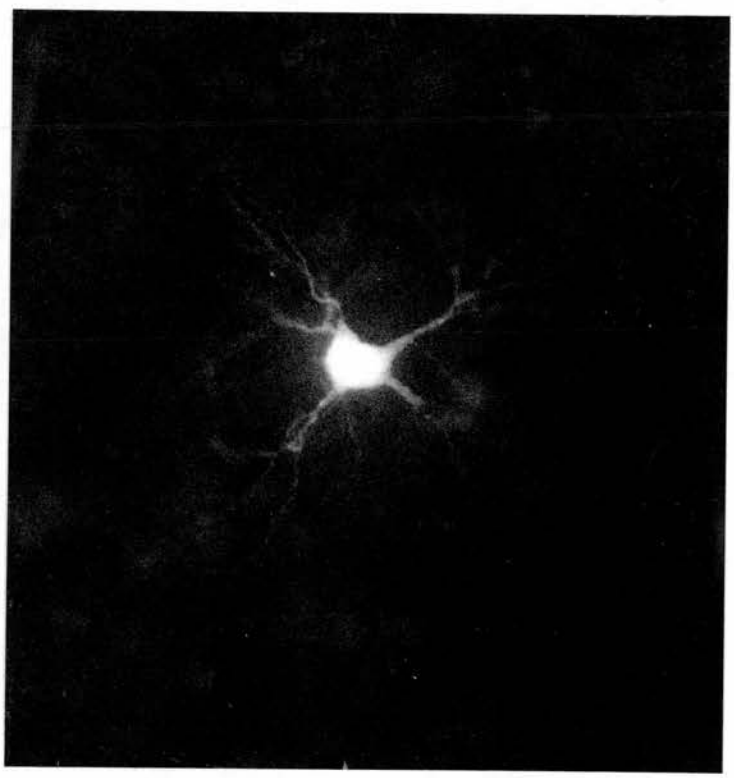
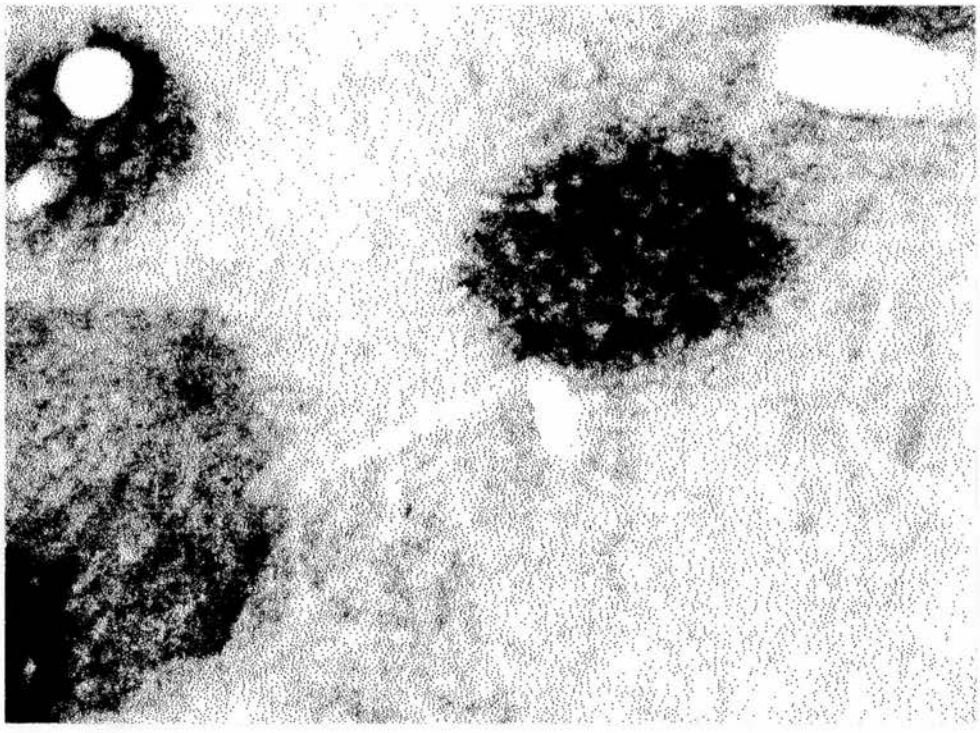


Figure 3.4(a) Computer scan of photograph of butyrylcholinesterase-stained striosomes before drawing cells (dark area to lower left is partly due to uneven cutting of tissue); horizontal section



(b) Biocom drawing of striosome outlines (shaded in) and landmarks with Eutectics cell drawing superimposed at site of cell body [see (d)]

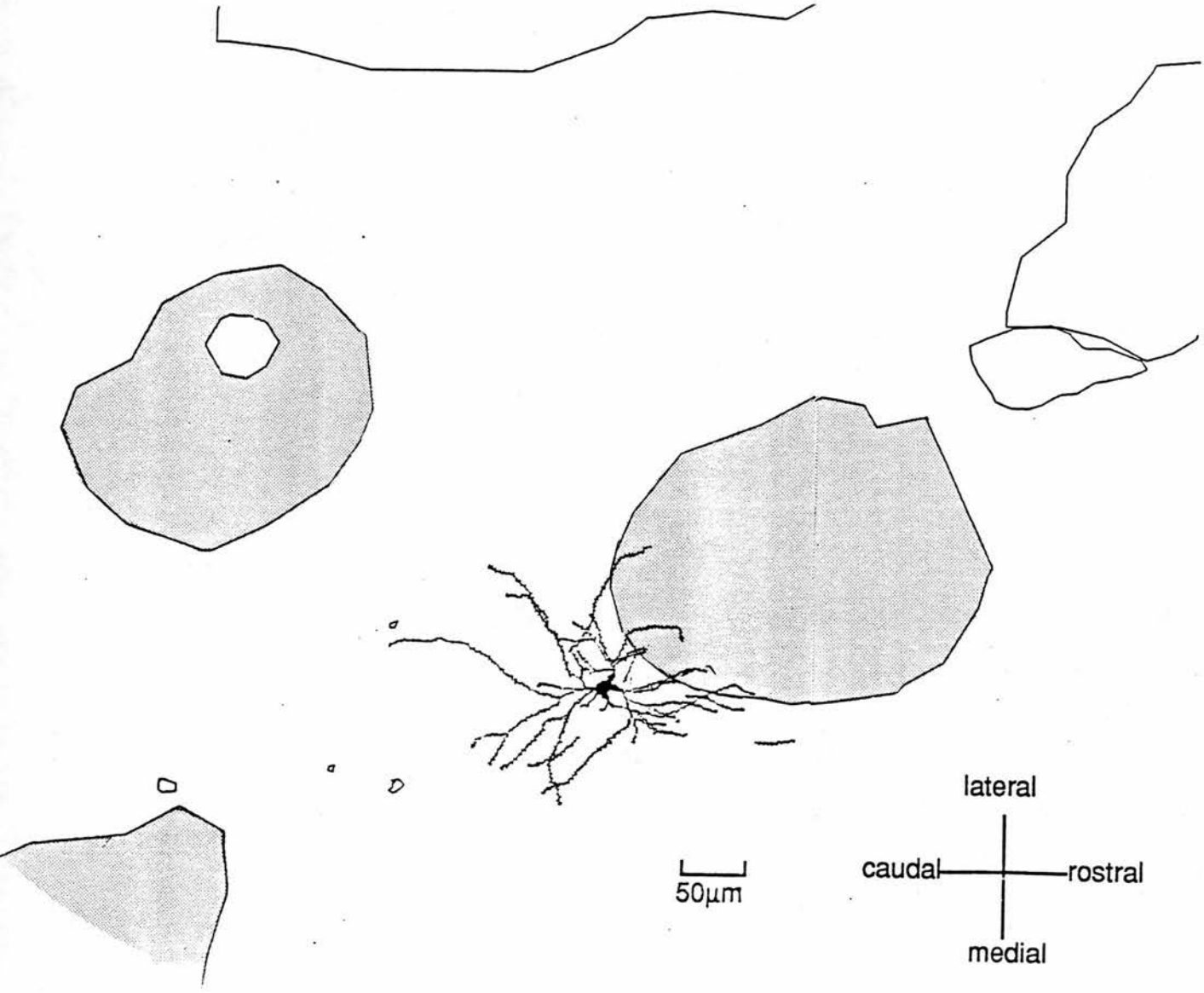
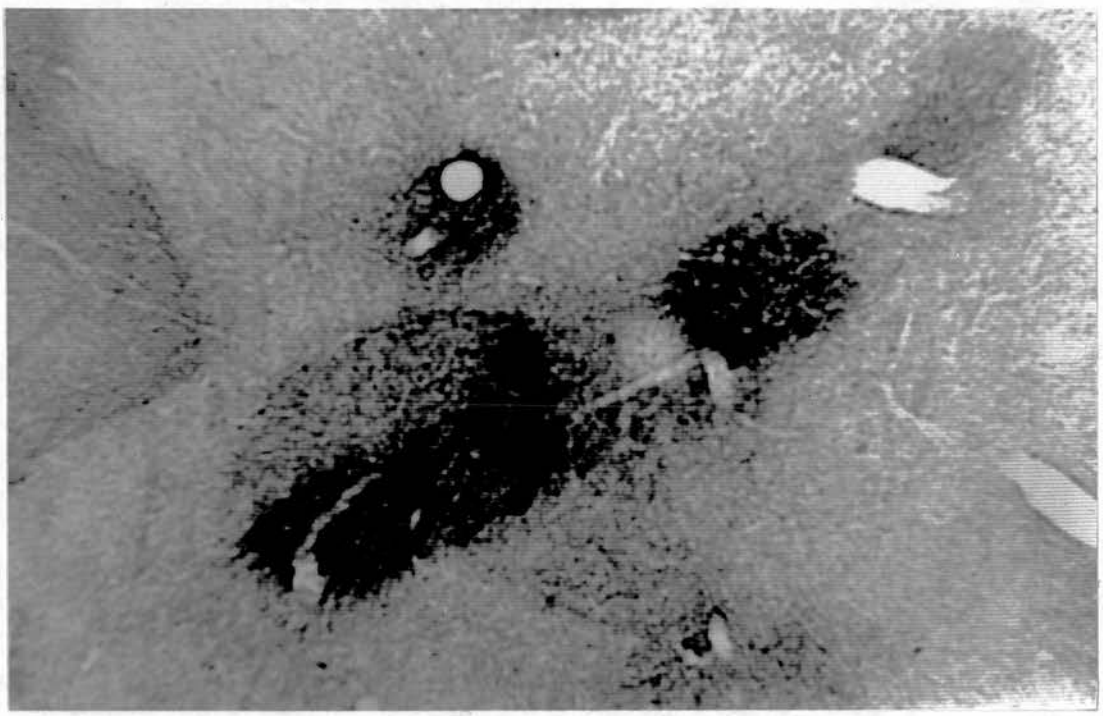


Figure 3.4(c) Photograph of same areas viewed on Biocom system screen. Note discoloured area where cell in (b) was drawn.

200µm



(d) As (c) with outlines of striosomes superimposed. Cell body outline can be seen in the centre of the discoloured area

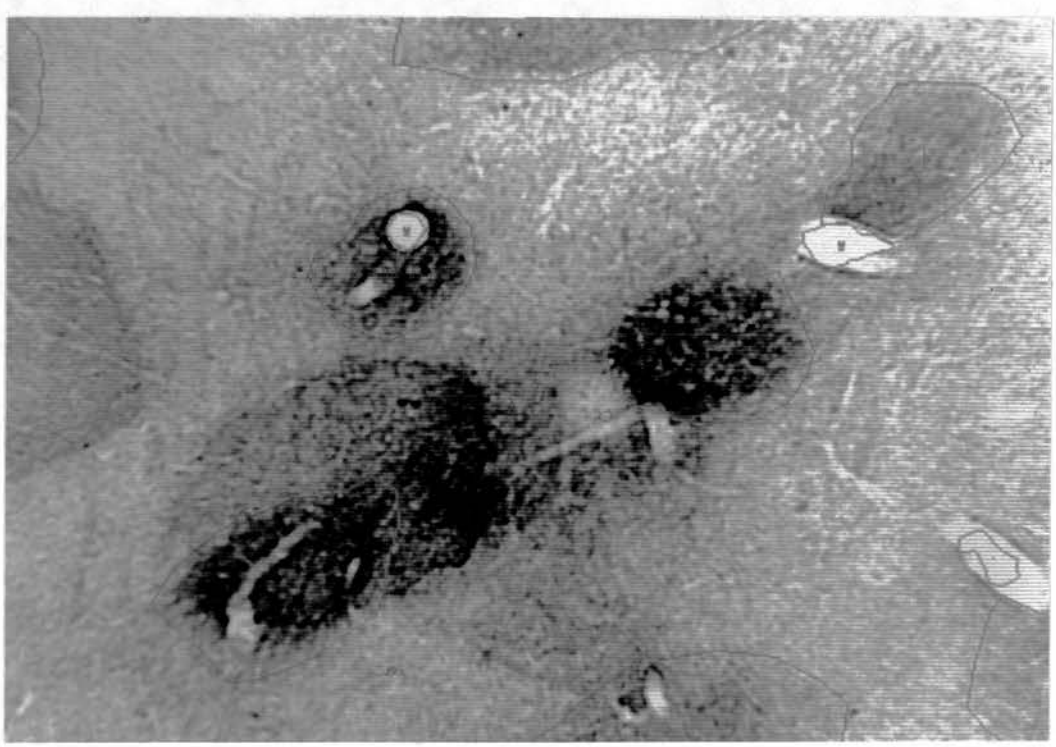


Figure 3.5 Cells with dendrites entering striosomes

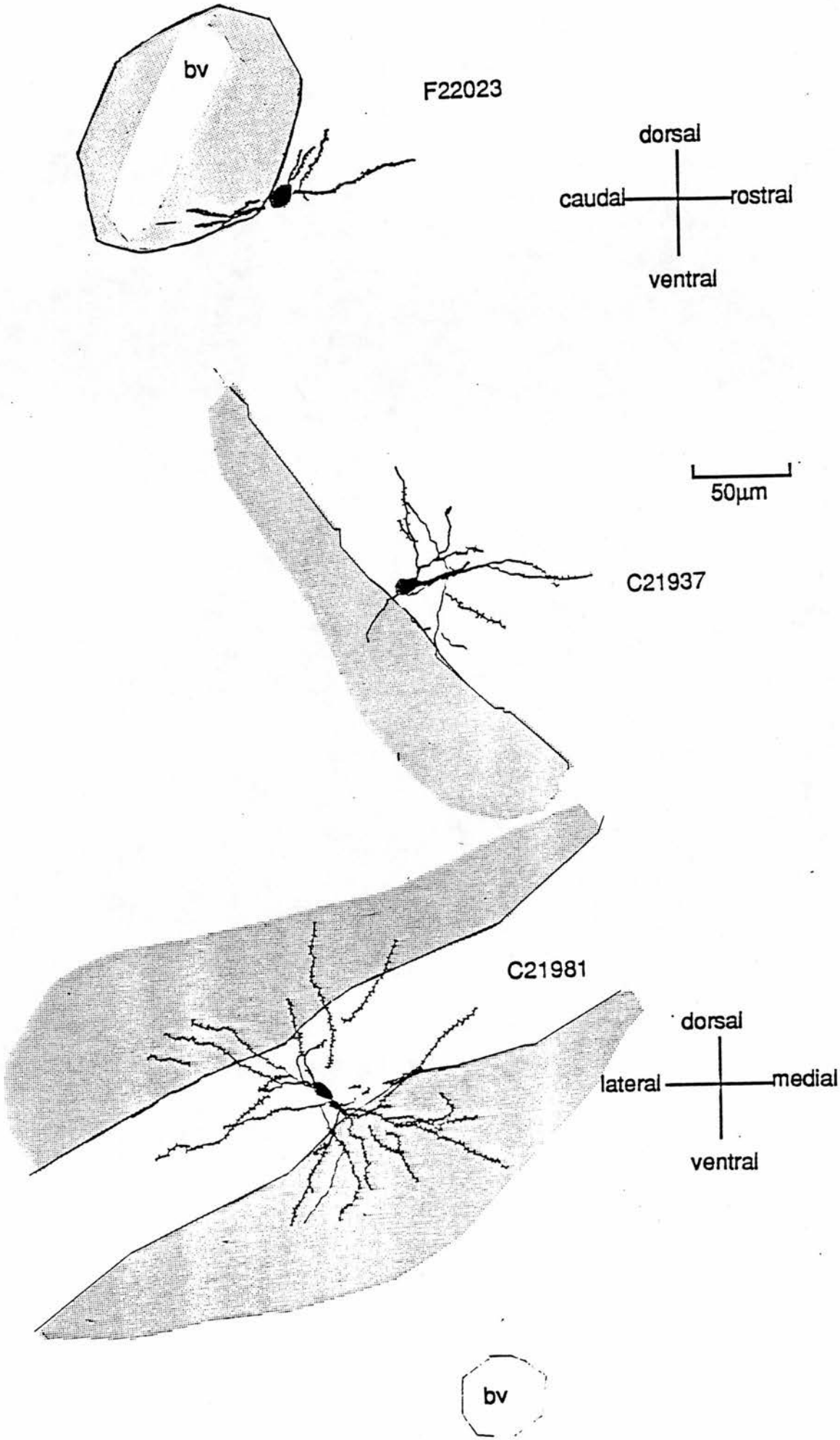

bv
F22023
dorsal
caudal
rostral
ventral
50µm
C21937
C21981
dorsal
lateral
medial
ventral
bv

Figure 3.6 Cells in matrix not near striosomes

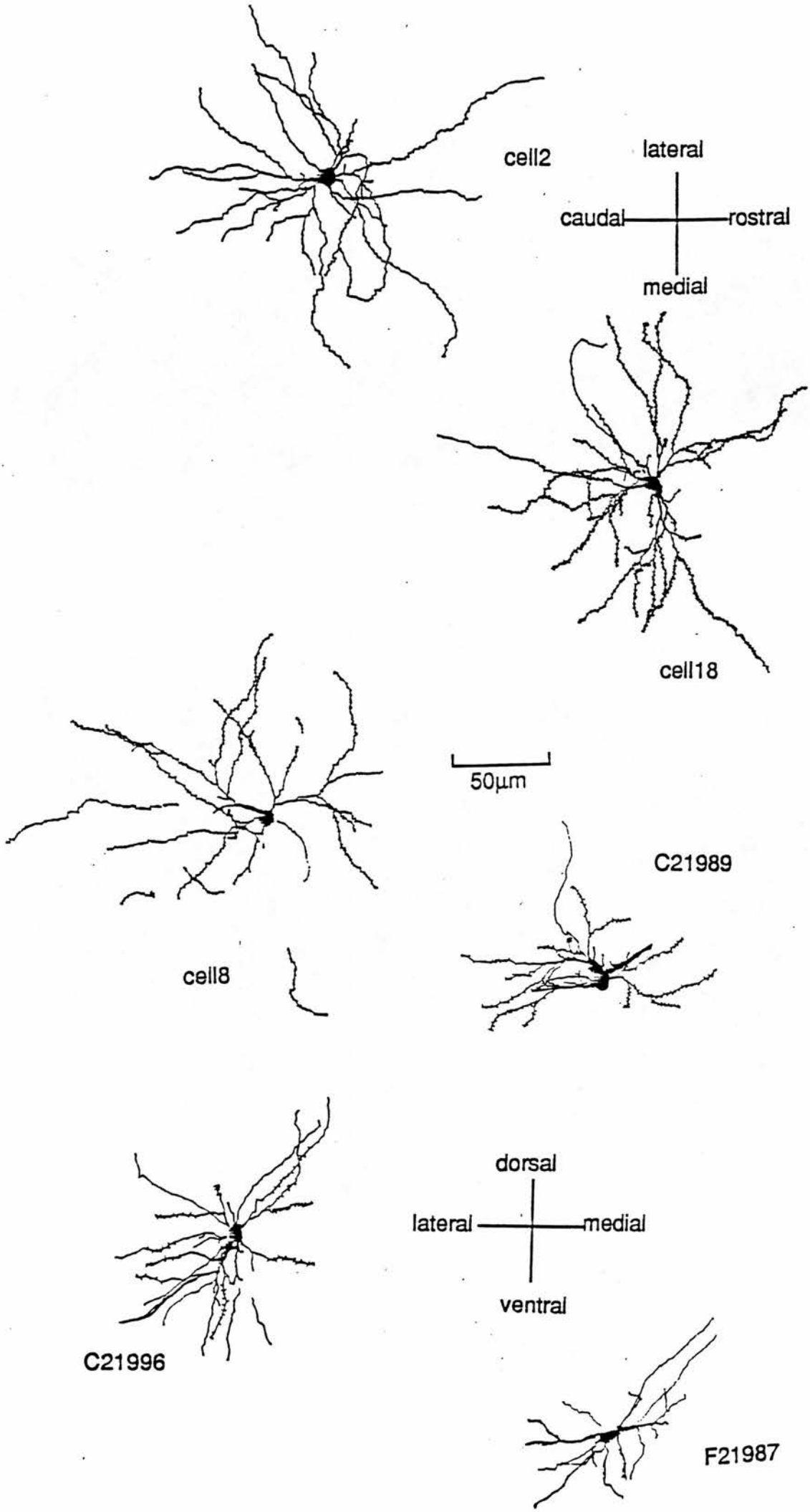

cell2
lateral
caudal
rostral
medial
cell18
50µm
C21989
cell8
dorsal
lateral
medial
ventral
C21996
F21987

Figure 3.7 Cells in matrix with distal tips of dendrites entering striosomes

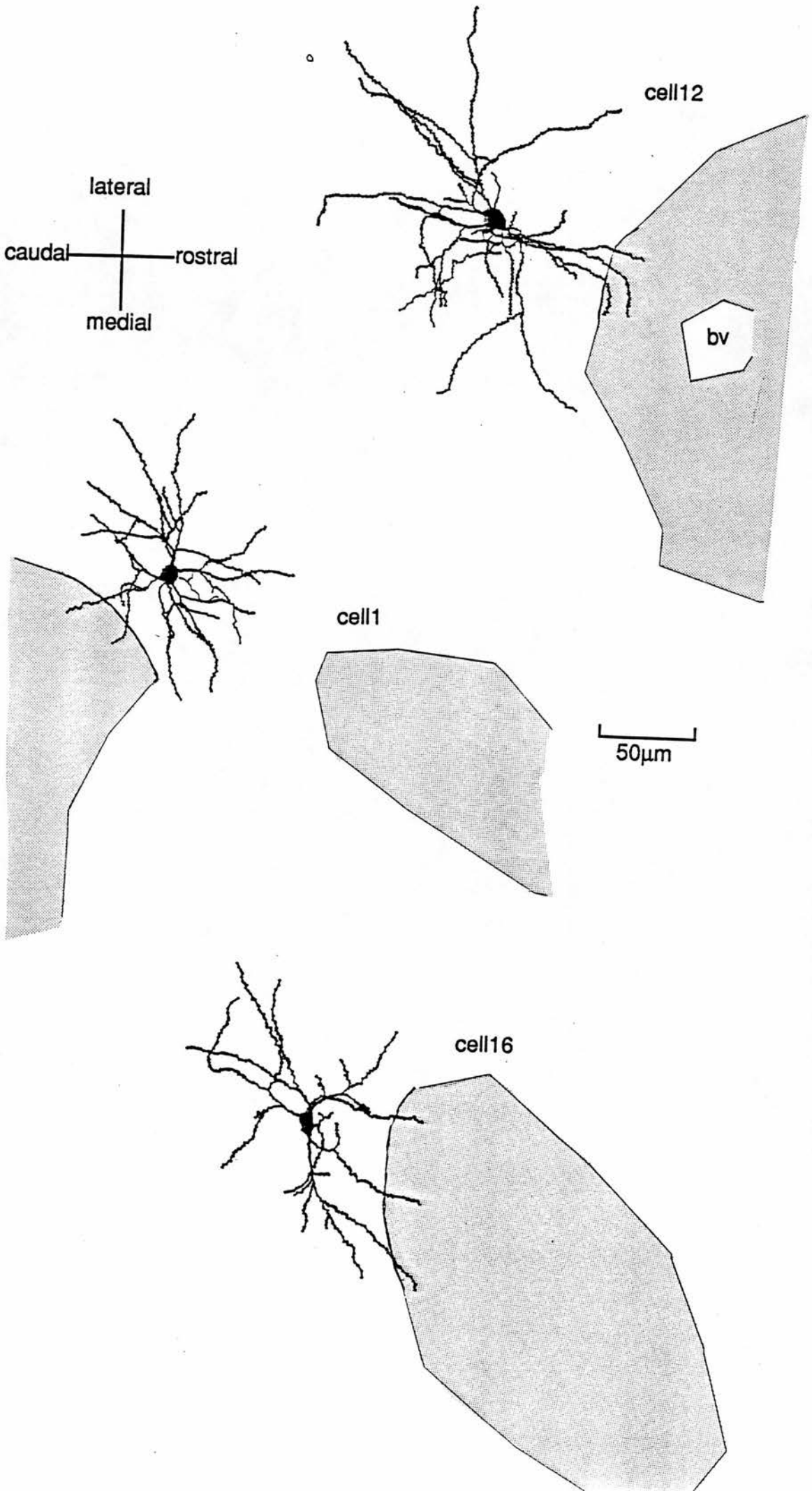
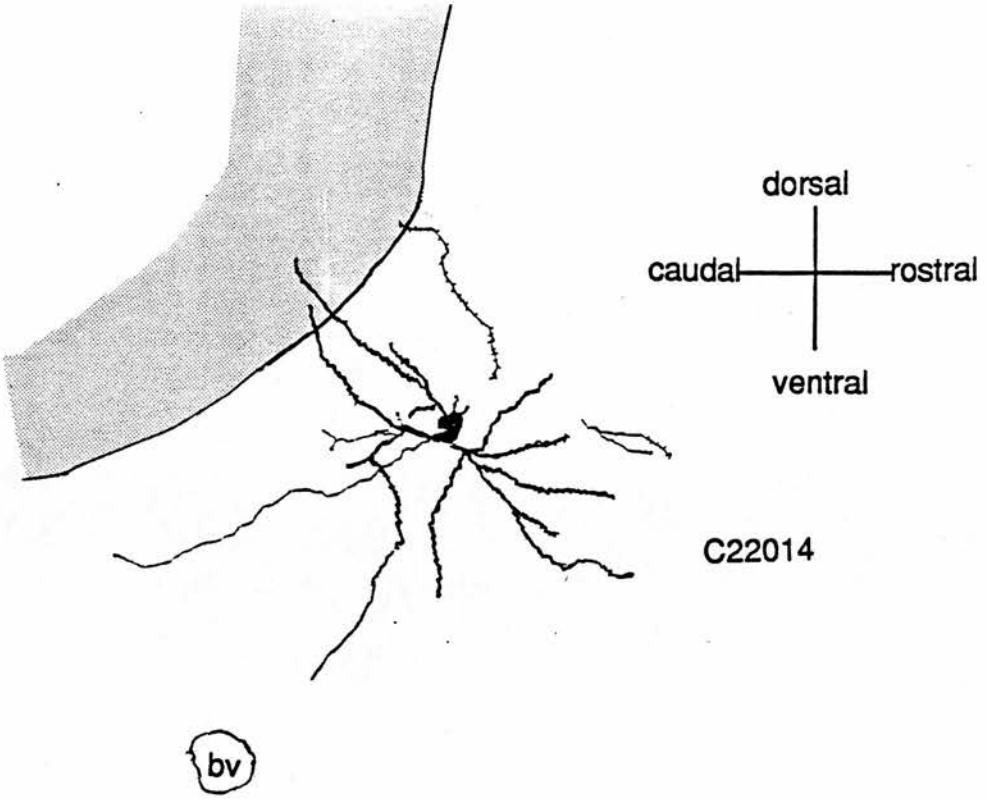
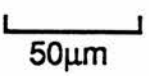

lateral
caudal
rostral
medial
cell12
bv
cell1
50µm
cell16

Figure 3.8 Cells in matrix with distal tips of dendrites entering striosomes


dorsal
caudal
rostral
ventral
C22014
bv


50µm

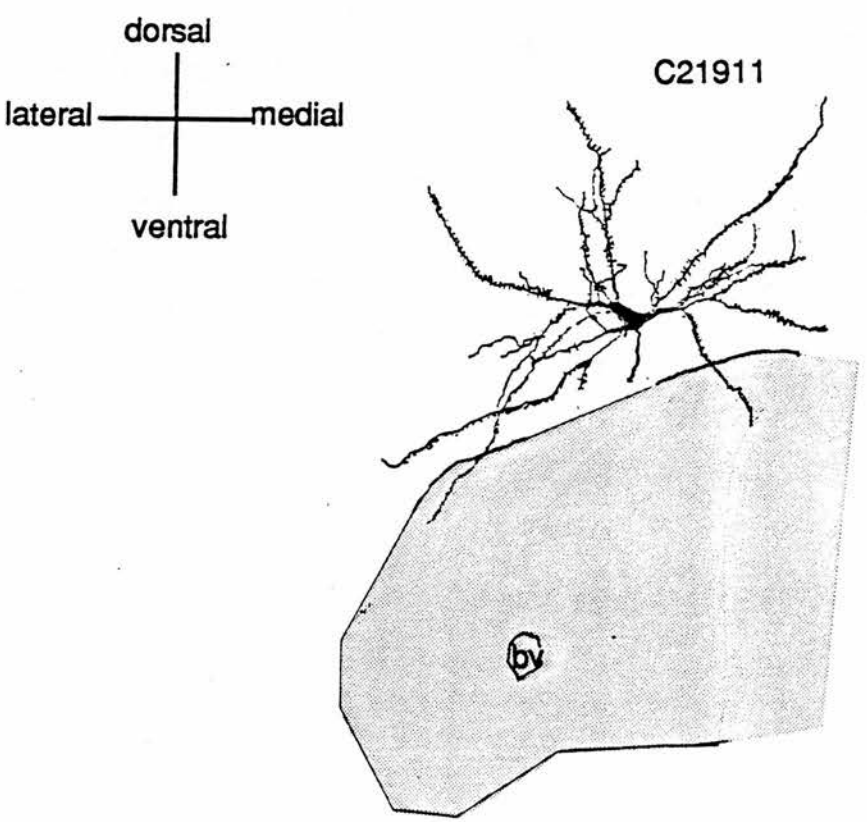

dorsal
lateral
medial
ventral
C21911
bv

Figure 3.9 Cells with dendrites outlining a striosomal border and sending dendrites towards a striosome

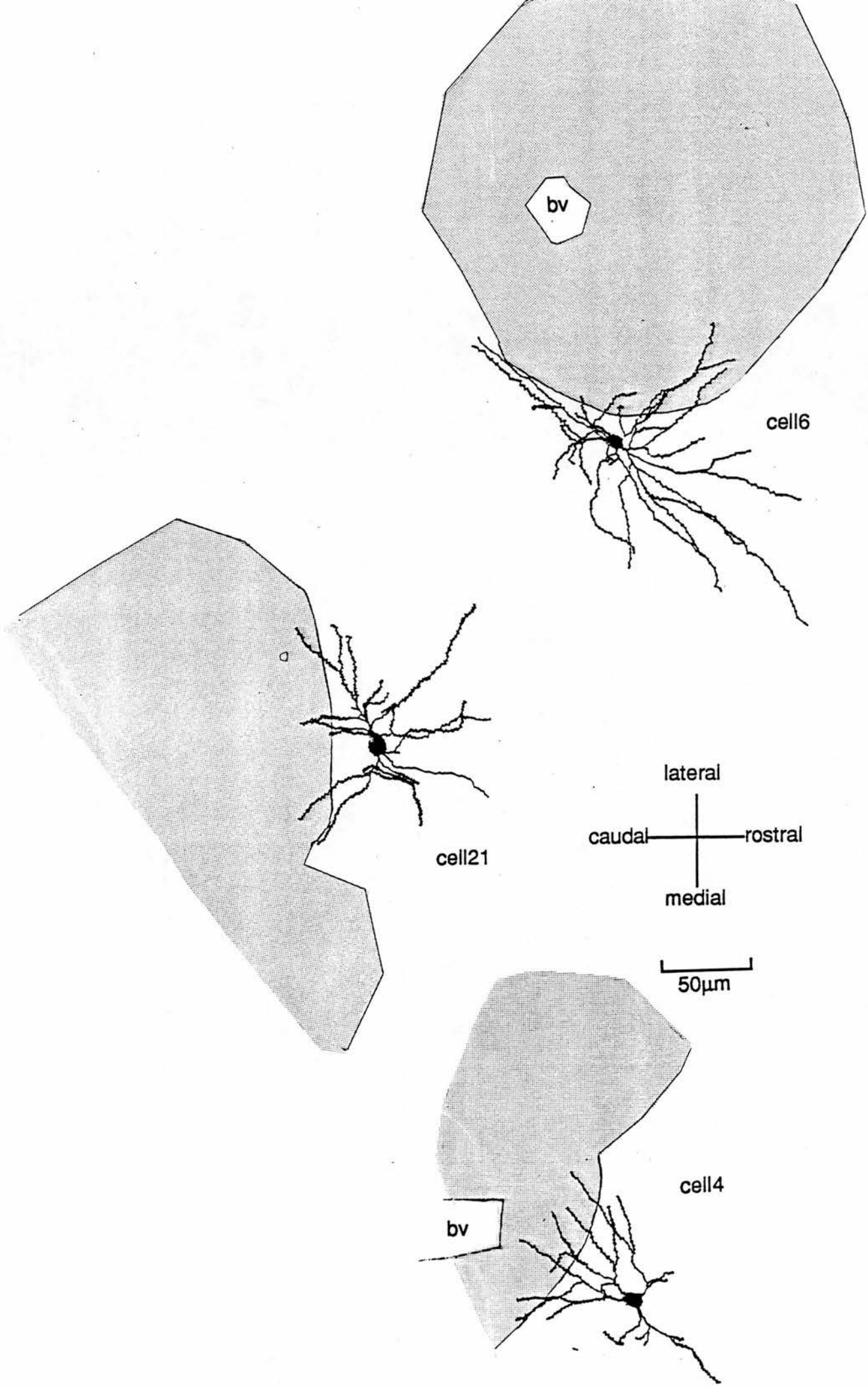

bv
cell6
cell21
lateral
caudal
rostral
medial
50µm
cell4
bv

Figure 3.10 Striosome cells with dendrites extending into the matrix

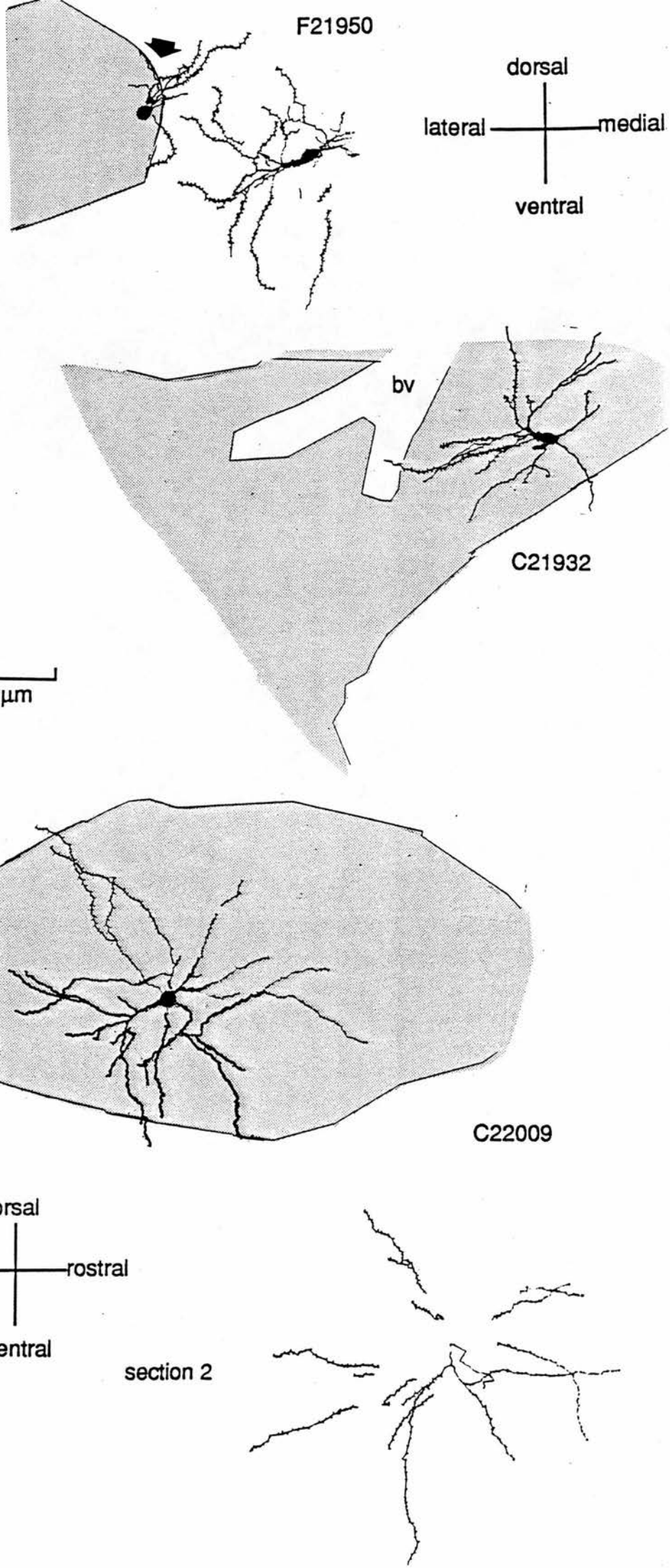

F21950
dorsal
lateral
medial
ventral
bv
C21932
50µm
section 1
C22009
dorsal
caudal
rostral
ventral
section 2

Figure 3.11 Cell with dendrites staying within a striosome and cells located in border regions

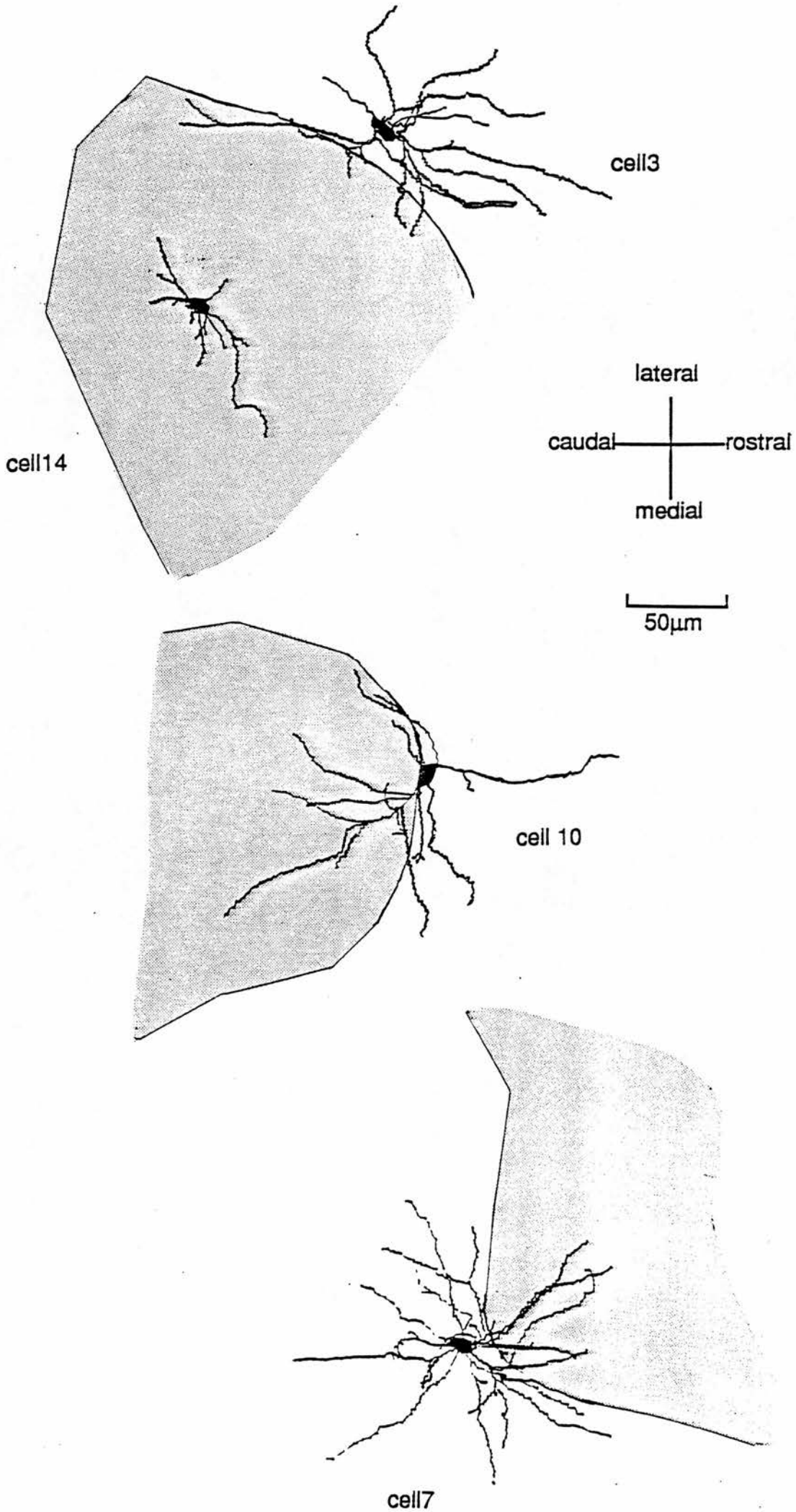
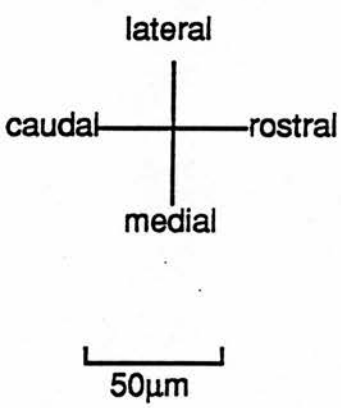

cell3
cell14
lateral
caudal
rostral
medial
50µm
cell 10
cell7

Figure 3.12 Cell located in matrix with dendrites in striosome on adjacent section

section 1

cell20



50µm

section 2

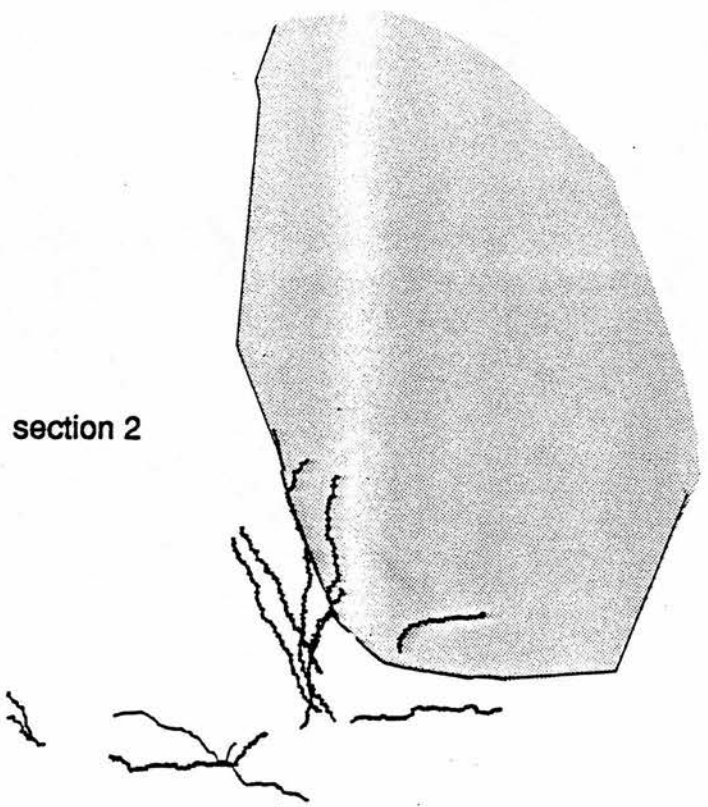


Figure 3.13 Large and medium sized aspiny neurons

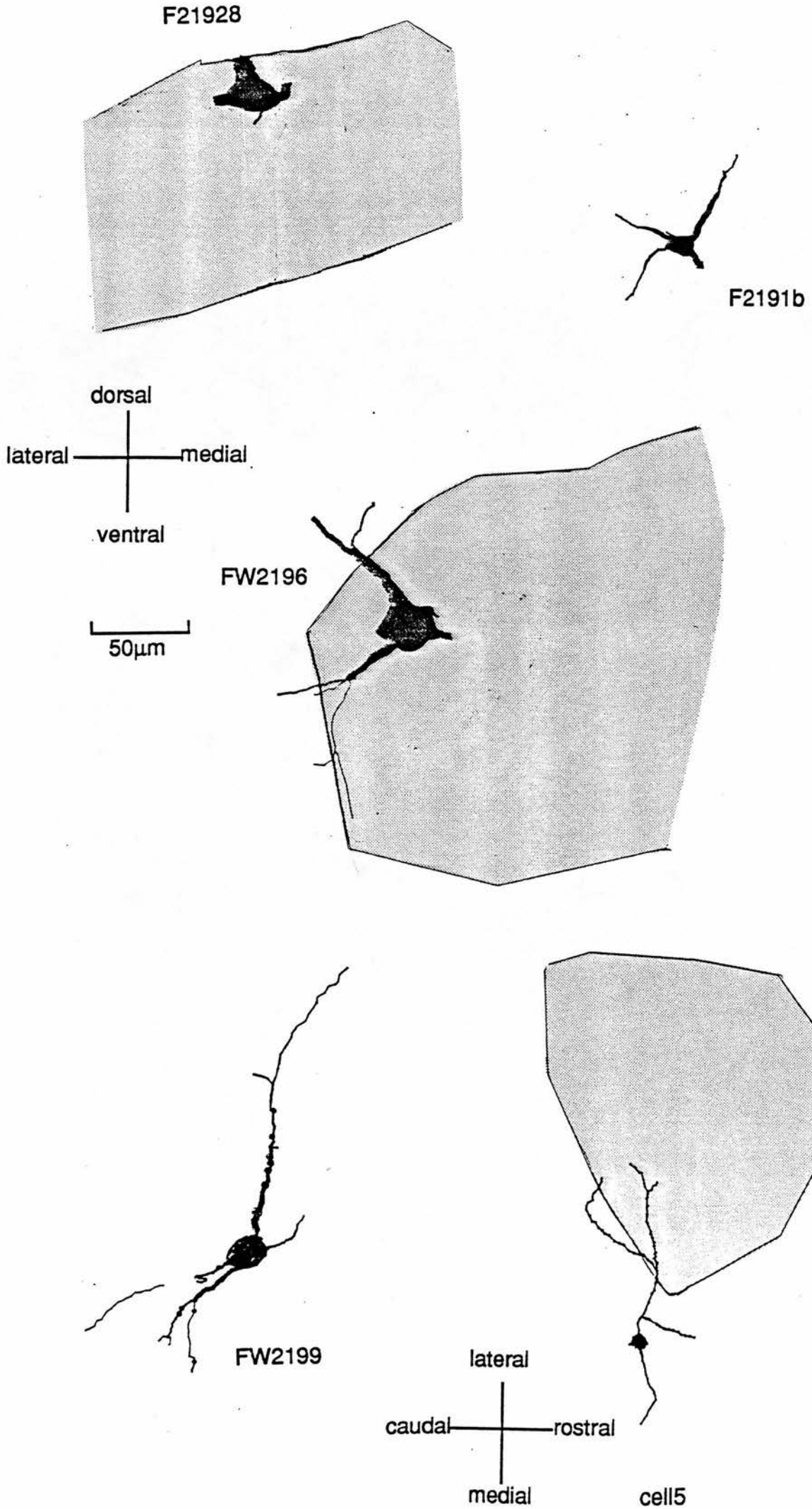

F21928
F2191b
dorsal
lateral
medial
ventral
FW2196
50µm
FW2199
lateral
caudal
rostral
medial
cell5

Figure 3.14(a) <u>All coronal cells (n=18)</u>

The mean value for dendritic length is represented for each 15^{o} segment.

From analysis of the cells in the coronal section, the mean dendritic length value for the latero-ventrolateral octant was significantly larger from all other directions, at a level of p=0.0001 by ANOVA.

The ventrolateral quadrant was significantly larger p=0.01.

The ventromedial quadrant was smaller p=0.007.

The ventromedioventral octant was significantly smaller at p=0.026.

Comparing complementary quadrants, ventrolateral and dorsomedial versus ventromedial and dorsolateral, they were different at p=0.047.

(b) [inset] Coronal section through ferret striatum with striosomes drawn on Biocom system, oriented as in orientation diagram

(c) Coronal section through ferret striatum with striosomes stained for butyrylcholinesterase, oriented as in orientation diagram

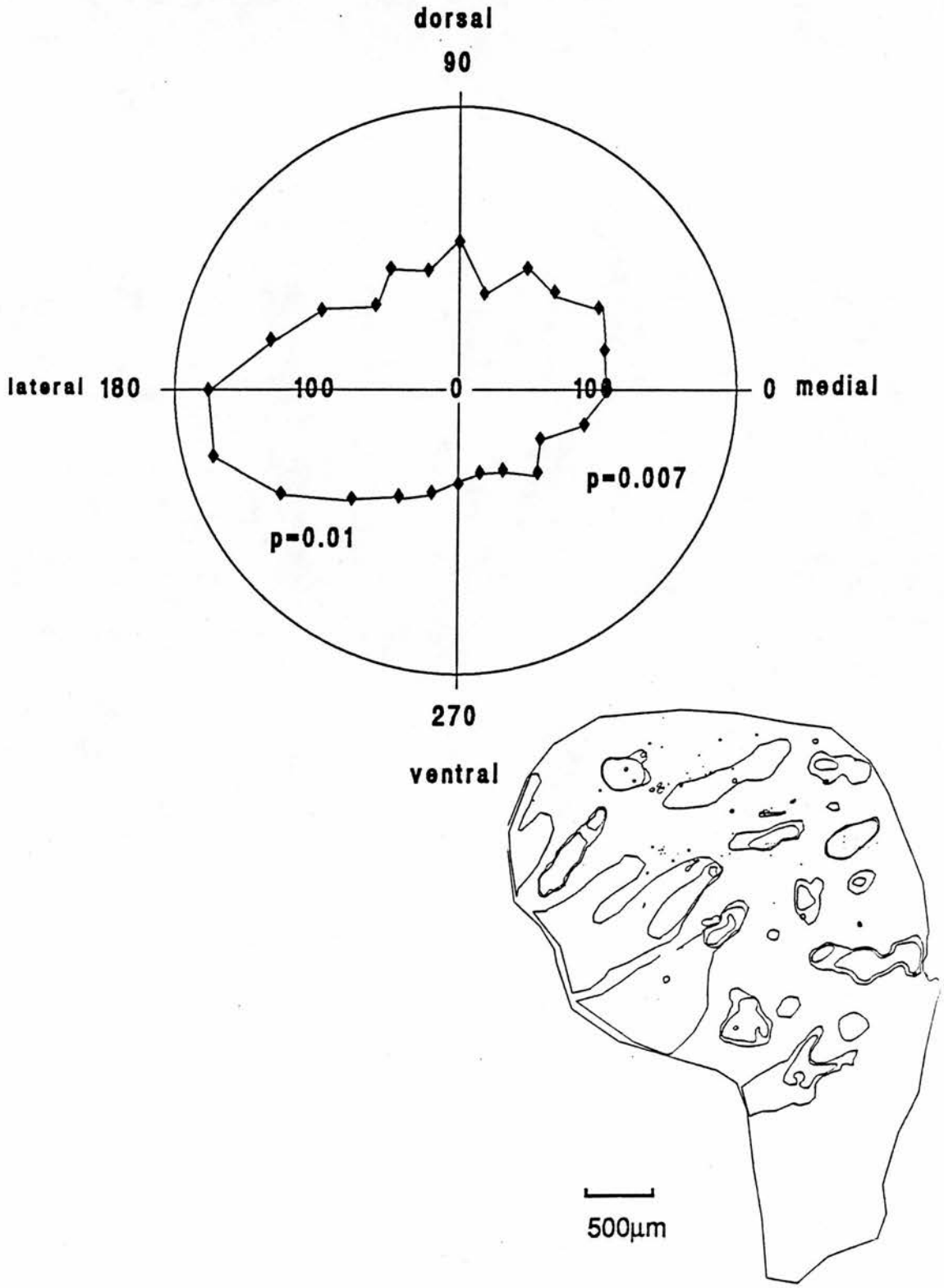

dorsal
90
lateral 180
100
0
100
0 medial
p=0.007
p=0.01
270
ventral
500µm

Figure 3.15 Cells not near striosomes (n=5)

The smaller values for this group are artefactual due to less good filling of some of these cells.

Probably due to the small sample size, only one octant was significantly different. This was the latero-ventrolateral octant, which was larger p=0.0003.

Complementary quadrants were significantly different p=0.018.

Cells near striosomes (n=13)

The ventrolateral quadrant was significantly larger p=0.01, and the latero-ventrolateral octant was significantly larger p=0.0001.

The ventromedial quadrant was smaller p=0.005, and the ventro-medioventral octant was significantly smaller p=0.022.

The ventromedial quadrant was smaller than its opposite quadrant p=0.0052. However, the overall orientation from comparison of complementary quadrants was not significant, apparently due to a larger component in the dorsolateral direction.

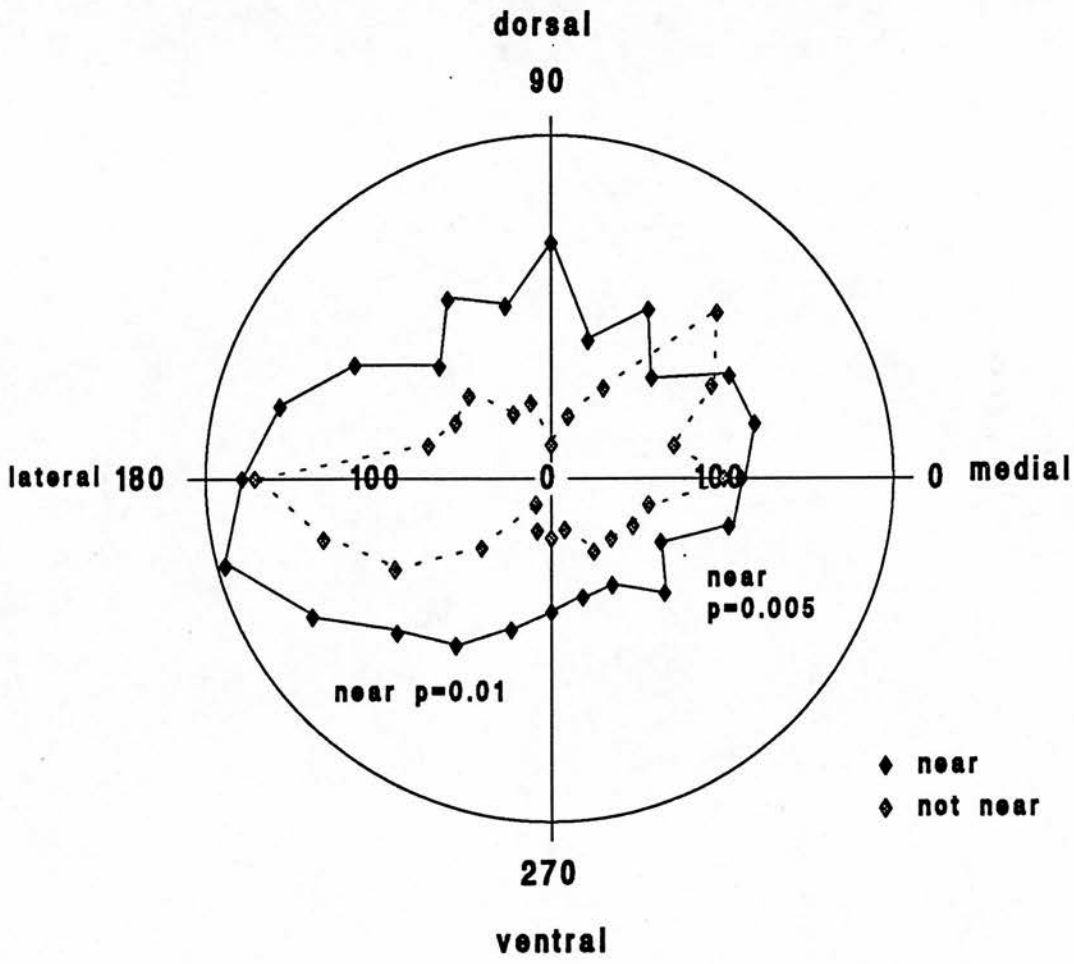

dorsal
90
lateral 180
100
0
100
0 medial
near
p=0.005
near p=0.01
near
not near
270
ventral

Figure 3.16 Cell bodies on deep sections (n=6)

The ventrolateral quadrant was significantly larger p=0.03.

The latero-ventrolateral octant was significantly larger than other directions, p=0.0001.

The opposite dorso-mediodorsal octant was also significantly larger, p=0.037.

The ventromedial quadrant was smaller than the dorsolateral quadrant p=0.039.

Cell bodies not on deep sections (n=12)

The ventrolateral quadrant was significantly larger p=0.04.

The latero-ventrolateral octant was significantly larger p=0.0002.

The ventromedial quadrant was smaller p=0.002, and the ventro-medioventral octant was smaller p=0.015.

The ventromedial quadrant was smaller than its opposite quadrant p=0.01, and complementary quadrants were significantly different p=0.019.

The dendritic values were longer for the deeper cells by paired t test p=0.0026.

Cells with their cell bodies on deeper sections would have more complete dendritic trees. The cells from more superficial sections appear to have fewer/less dendrites in the ventromedial direction. From the horizontal data, the shorter medial component is in a rostral direction. From the way the slice was cut, cells were filled on the rostral face, and thus one would expect a loss of rostral dendrites of more superficial cells.

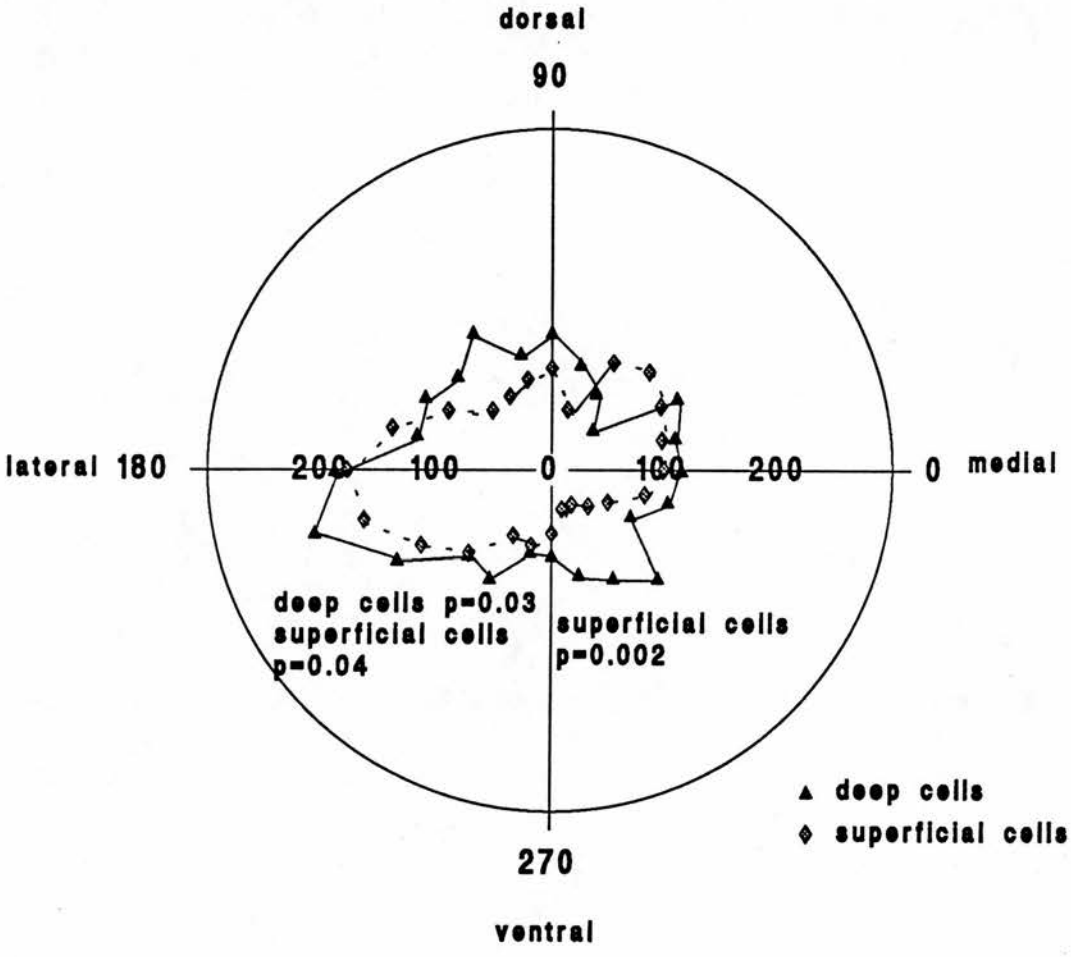

dorsal
90
lateral 180
200
100
0
100
200
0
medial
deep cells p=0.03
superficial cells
p=0.04
superficial cells
p=0.002
deep cells
superficial cells
270
ventral

Figure 3.17(a) <u>All horizontal cells (n=21)</u>

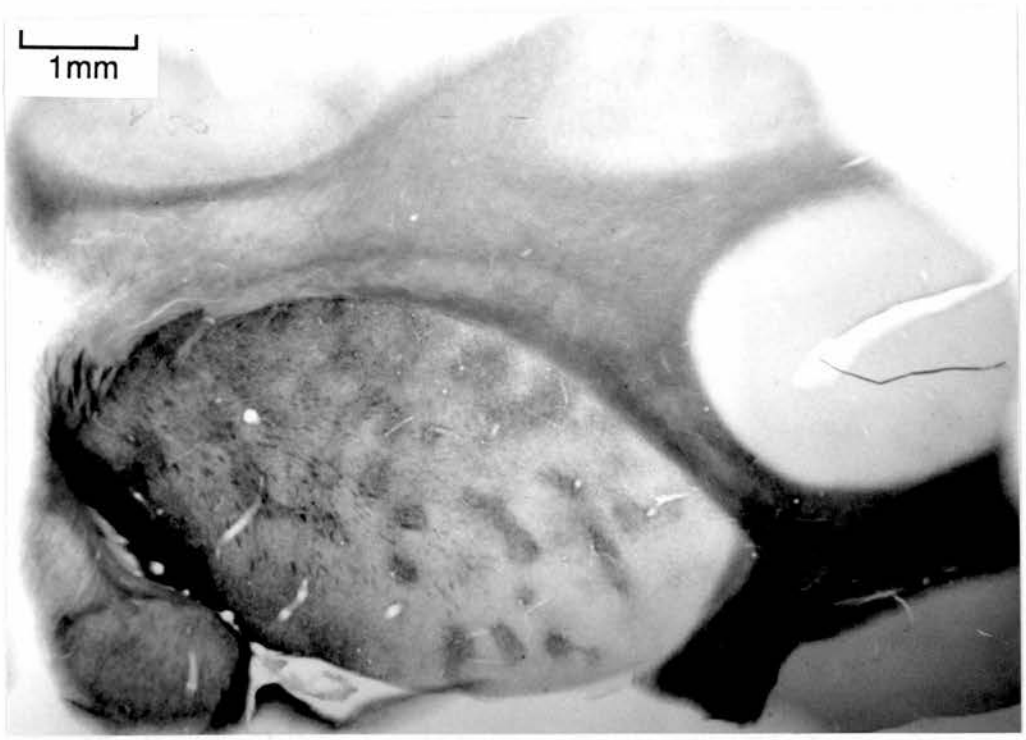
From analysis of the horizontal section, the rostrolateral quadrant was significantly larger p=0.012, and the latero-rostrolateral octant was significantly smaller at a level of p= 0.017.

The rostrolateral quadrant was significantly smaller than the opposite caudomedial quadrant p=0.035.

The caudolateral quadrant was larger p=0.004, and the caudo-caudolateral octant was significantly larger at a level of 0.001.

The complementary quadrants (caudolateral and rostromedial v. rostrolateral and caudomedial) were significantly different p=0.0009.

(b) Horizontal section stained for BChE, oriented as in orientation diagram



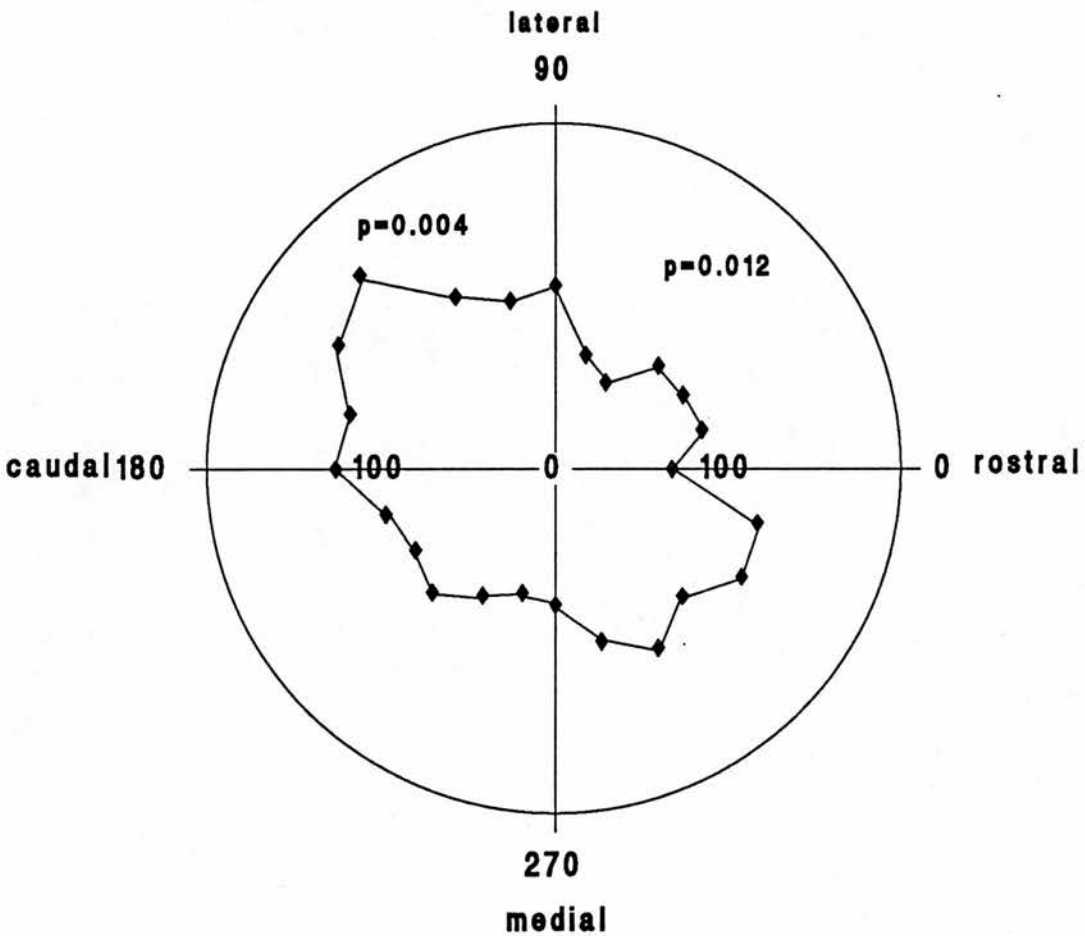

lateral
90
p=0.004
p=0.012
caudal 180
100
0
100
0 rostral
270
medial

Figure 3.18 Cells not near striosomes (n=4)

Despite the small n for these cells, there was strong evidence of orientation.

The rostrolateral quadrant was significantly smaller p=0.01.

The rostrolateral quadrant was smaller than the opposite quadrant p=0.0043.

The caudolateral quadrant was significantly larger p=0.0001, as was the laterocaudolateral octant.

The caudolateral quadrant was significantly larger than the opposite quadrant p=0.013. The complementary quadrants were significantly different p=0.027.

Cells near striosomes (n=17)

The rostrolateral quadrant was significantly smaller, p=0.006, and the laterorostrolateral octant was significantly smaller p=0.034.

The caudolateral component was less striking, with the caudal octant significant at p=0.02, and the quadrant not significant (p=0.069).

The complementary quadrants were different p=0.0024

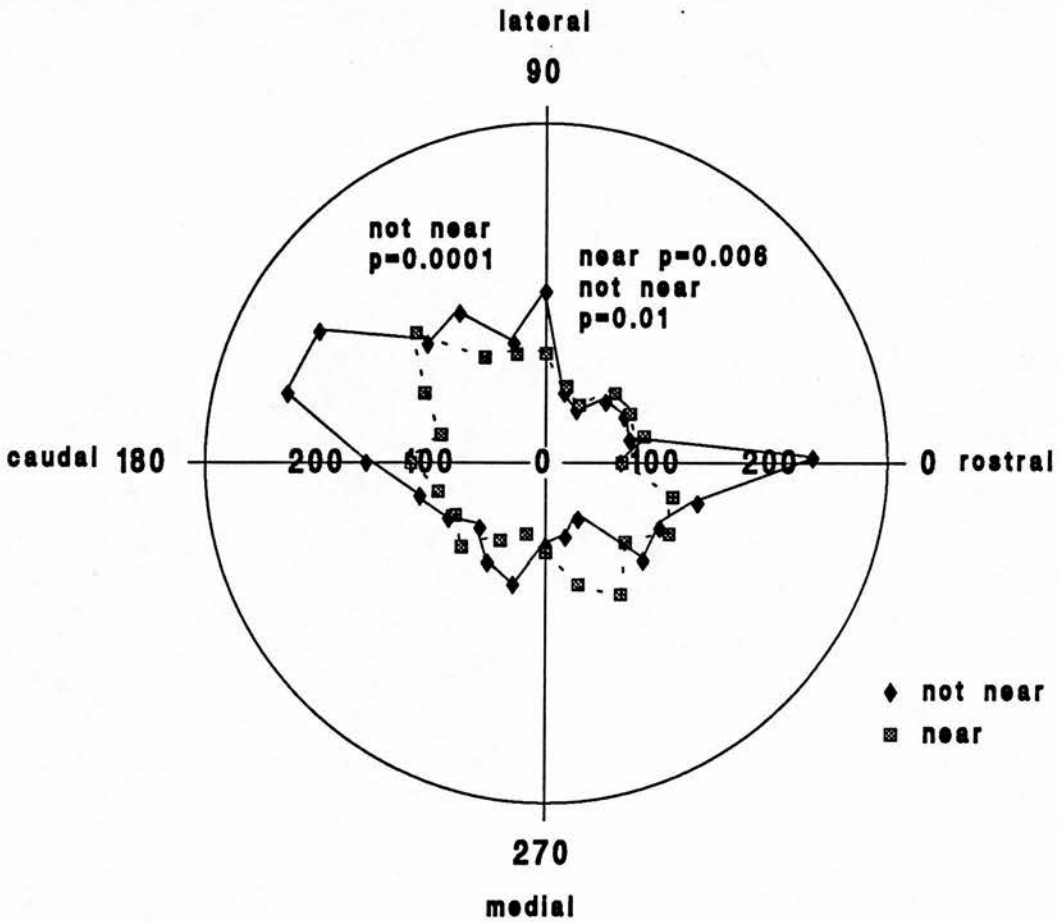

lateral
90
not near
p=0.0001
near p=0.006
not near
p=0.01
caudal 180
200
100
0
100
200
0 rostral
not near
near
270
medial

Figure 3.19 Cell bodies on deep sections (n=8)

The rostrolateral quadrant was significantly smaller, p=0.005, and the latero-rostrolateral component was marginally significantly smaller p=0.047.

The rostrolateral quadrant was smaller than the opposite quadrant p=0.0016.

The caudolateral quadrant was larger p=0.04, and the caudo-caudolateral octant was significantly larger p=0.0024

The complementary quadrants were not different.

Cell bodies not on deep sections (n=9)

The rostrolateral quadrant was smaller p=0.01, and the latero-rostrolateral octant was significantly smaller p=0.045.

The caudolateral quadrant was significantly larger p=0.007, and the caudo-caudolateral octant was larger p=0.015.

The complementary quadrants were significantly different p=0.0001.

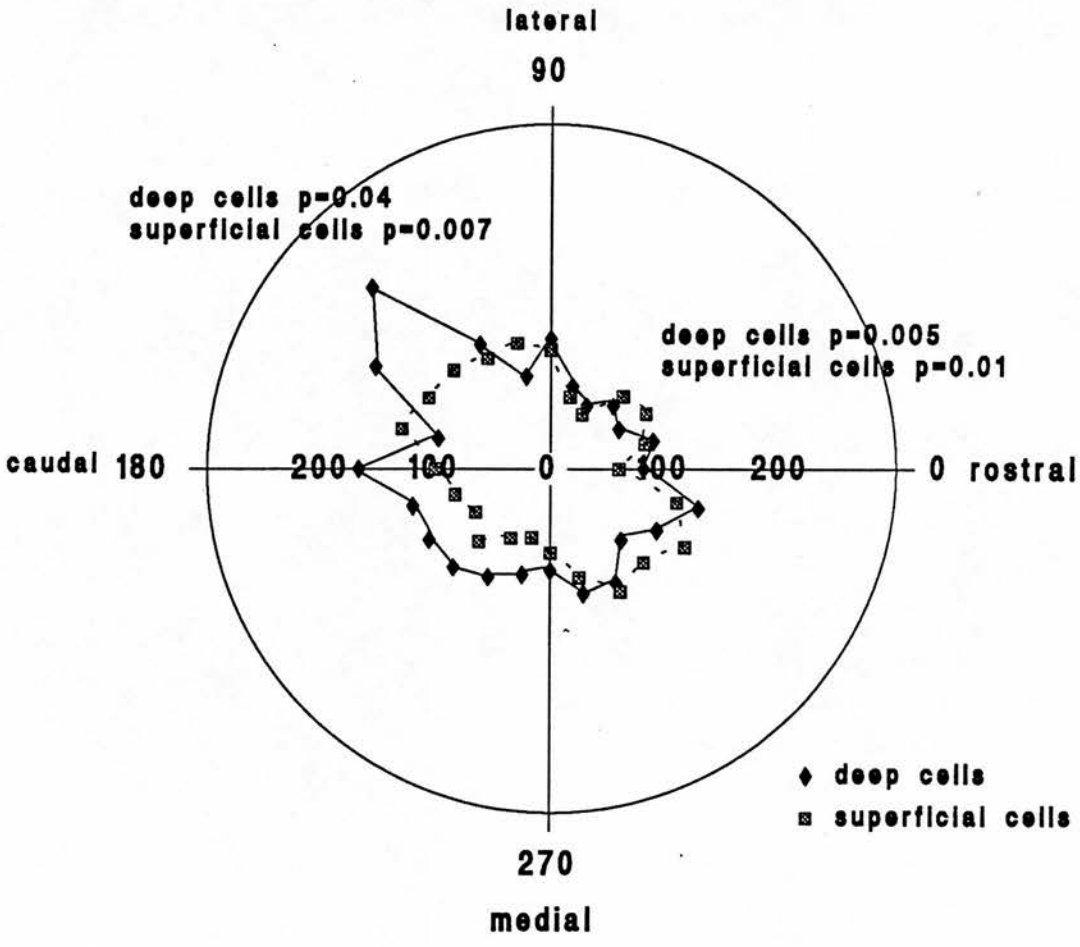
As would be expected, the dendritic values were significantly longer for the cells on deeper sections by paired t test p=0.036

The differences between the deeper and the more superficial cells were seen mainly in the decrease in significance of the caudolateral quadrant in the deeper cells.

From the figure, this would appear to be due to larger values in the caudomedial component of deeper cells.

This would suggest that these components were under-represented in the more superficial cells.

From the coronal cell data, the shorter medial component is in the ventral direction. Cells were filled on the dorsal side of the slice, and thus dendrites in the dorsal direction would be under-represented. Thus this difference does not appear to be due to a bias in the experimental method.


lateral
90
deep cells p=0.04
superficial cells p=0.007
deep cells p=0.005
superficial cells p=0.01
caudal 180
200
100
0
100
200
0 rostral
deep cells
superficial cells
270
medial

Chapter 4

Studies of striatal cells in the primate

Introduction

A major problem with the technique used in the preceding experiments is that the fluorescent dye is not permanent and will fade with time although this can be minimised by keeping the slides at -70°C. Even more of a problem is that it fades with exposure to light, especially during drawing of the cells under fluorescent illumination. The butyrylcholinesterase label becomes darkened during this process, making striosome borders less readily identifiable. Thus was sought a permanent photodense cell label which could be combined with a striosome marker.

This was particularly important for the experiments to be carried out in the primate, as both the tissue and the results were more valuable, in terms both of cost and relevance to human pathophysiology.

Experiments basically similar to those in the ferret were carried out in the squirrel monkey, in order to provide results which would correlate more with human striatal function.

There were two ways in which the method could be modified to produce a permanent cell marker. One would be to change the label used to fill the cells to one which would produce a photodense reaction product. The second was to retain the use of lucifer yellow, and to

find a way of making it opaque to visible spectrum light.

The major advantage of using a fluorescent dye, and thus emitted (as opposed to transmitted) light, to see the micropipette and the filling cell, is that light does not have to pass all the way through the tissue slice to reach the microscope objective. The slice and pipette could be illuminated from above, rather than the light having to pass through the slice before reaching the microscope objective. It was also necessary to have some way of telling if the pipette has penetrated a cell, and as the set-up involved a fixed rather than a living slice, the ability to see the pipette filling the cell was an absolute necessity. For these reasons the use of a fluorescent dye was retained. The options were to use a different fluorescent dye which could in some way produce a photodense reaction product, to use the lucifer yellow to see the pipette and cell but mixed with a label which could be later reacted, or to continue to use lucifer yellow as the sole label, but to change it or label it so that it would be visible under visible spectrum light.

Either technique would have to be compatible with a striosome label, such as a stain for cholinesterase (acetyl- or butyryl-) or immunohistochemistry to substances such as enkephalin, calcium binding protein, or tyrosine hydroxylase.

Photoconversion

Since it was simple to obtain good quality fills with lucifer yellow (LY) this technique was tried initially. This method involves exposing LY to fluorescent illumination (Sandell and Masland, 1987).

Method

Cells were filled with lucifer yellow in lightly-fixed slices of rat or ferret striatum, as prepared in the previous chapter. The reaction was tried on the whole slice, on post-fixed 50um sections, and before or after the butyrylcholinesterase reaction (see previous chapter).

A drop of 3,3'-diaminobenzidine (DAB) solution (1.5mg/ml in 0.1M phosphate buffer) was placed upon the tissue; after a preincubation of 5-10 minutes a field containing a LY-filled cell was illuminated on the microscope stage with fluorescent light using the FITC filter, using either a 10x or a 50x long working distance objective, until all fluorescence was extinguished. Whenever the DAB solution became discoloured, it was replaced with fresh solution.

In order to intensify the reaction product, and to improve labelling of fine processes and spines, a section which had been photoconverted was incubated in osmium 1% in phosphate buffer.

Results

When the whole slice was used, there was inadequate penetration of the DAB to the cell, and after a long exposure time there was still some visible fluorescence. More success was had with 50um sections, but the BChE reaction was prevented by prior exposure to DAB. When photoconversion was tried on sections which had been already labelled for BChE, the field exposed became darkened, probably by DAB complexing with the ferricyanide reaction product.

The labelling produced by photoconversion was usually not very satisfactory, with discolouration of the overlying tissue, and inadequate definition of all cell processes. Osmification caused the whole section to darken without useful intensification of the labelled cell.

This process was also limited by the fact that in order to photoconvert all distal processes of filled cells, of which there might be a large number in any given slice, both surfaces of every section would have to be exposed, which was impractical with the equipment available .

Biocytin

Biocytin is a small molecule (molecular weight 372.5) which has been used as an intracellular label in

electrophysiological slice experiments (Horikawa and Armstrong, 1988; Kawaguchi *et al.*1989, 1990). It is highly soluble in water and easy to inject with current pulses. It can readily be reacted with avidin-HRP (Vector) to produce a permanent dark reaction product after incubation with hydrogen peroxide and DAB.

Method

A solution of 4% biocytin (Sigma) (Horikawa and Armstrong, 1988) in water was used in the micropipette. Biocytin behaves like a simple amino acid, and is positively charged in water.

In order to see the micropipette under the fluorescent microscope it was necessary to mix it with LY. Initially it appeared that the different charges of the two molecules would make it difficult to eject adequate amounts of LY, although it soon became clear that only a trace of LY was necessary to make the pipette and the impaled cell visible. In the protocols described for electrophysiological experiments various solutions e.g. of 0.5M potassium chloride in 0.05M tris buffer, were used, however it soon became clear that this was not important.

After cell filling, the tissue was post-fixed and sectioned as usual.

To develop the dye, the sections were incubated for one

hour in ABC solution (avidin-biotin complex: Vector labs) 1% in 0.1M phosphate buffer with 0.5% Triton X (Sigma). The detergent was essential for penetration of label into the tissue. After several washes the sections were developed in 0.06% diaminobenzidine and 0.003% hydrogen peroxide in 0.1M phosphate buffer.

Alternatively the sections were incubated for 30 minutes with 5% streptavidin-alkaline phosphatase (Vector) in 0.01M tris/saline buffer, and then in the alkaline phosphate substrate kit II (Vector) in 100mM tris HCl pH 9.5. This also labels endogenous peroxidase in blood vessel walls, so an inhibitor of endogenous peroxidase, 1mM levomisole (24mg/100ml), was necessary.

Various striosomal labels were tried in combination with biocytin, such as histochemistry for butyrylcholinesterase (see previous chapter) and immunohistochemistry to enkephalin (1:1000, Dr R.P. Elde).

Results

Initial results were hopeful, once conditions were such that a significant amount of biocytin was being ejected. Dendrites labelled with reaction product were seen. However it soon became evident that the biocytin leaked out of the cell, even when the LY did not appear to. Whilst distal dendrites could be clearly seen, the

details around the cell body were obscured. Regardless of variations in filling technique, current polarity, or filling time, there was always leakage around the cell body which made it impossible to determine proximal detail. This technique was therefore abandoned.

Fluorochrome-labelled horseradish peroxidase

Horseradish peroxidase (HRP) is used to fill cells in intracellular electrophysiological experiments, but has also been shown to diffuse satisfactorily along axons and dendrites in fixed tissue (Kageyama and Meyer, 1987). Fluorescein- and rhodamine-labelled horseradish peroxidase are fluorescent molecules, which were visible in the micropipette, and were useful in order to tell whether the dye was entering the cell. The necessity of changing the filter cube during cell filling made the use of the rhodamine-labelled HRP impractical. The fluorescein-labelled HRP was clearly visible in the pipette, but it flowed sluggishly from the tip when current was passed. The molecules are much larger than LY and less soluble in water and frequently the dye blocked the pipette tip. This method was abandoned.

Antibody to Lucifer Yellow

Finally success was achieved with an antibody raised in rabbit to lucifer yellow (gift of Drs. Kuwada and

Knapfl) (Taghert *et al.*1982). The protocol was ultimately carried out in squirrel monkeys, and is here described in its entirety. In order to label striosomes, both immunohistochistry and cholinesterase staining were tried.

Method

Slice preparation

An adult squirrel monkey was anaesthetised initially with a dose of 0.3ml ketamine i/m, and then given a lethal dose (0.3ml) of Nembutal. The descending aorta was clamped and the animal was perfused through the left ventricle with a prewash of 0.9% saline at room temperature until most of the blood was washed from the upper body circulation. It was then fixed with 500ml of fixative (2% paraformaldehyde, 0.1% glutaraldehyde in 0.1M phosphate buffer with 0.9% saline) at room temperature. The skull was opened and the dura removed; a block was made containing the striata whilst the brain was occasionally washed with chilled phosphate buffer. The halves of the brain were separated and the striata put in a beaker of chilled buffer for a couple of minutes. The bulk of the corpus callosum and other surrounding white matter was removed, but otherwise the striatum was left intact. One side was sliced coronally from rostral to caudal, and the other side was sliced sagittally from lateral to medial.

Four hundred micron thick slices were cut using very sharp blue steel blades, which were changed often during the cutting of each block as soon as they appeared to cut less well. The vibratome was set to cut at the slowest speed, with the widest amplitude of lateral movement. A block of agar was glued to the chuck to prevent the block of tissue deforming as the blade cut it. The vibratome bath was filled with chilled 0.1M phosphate buffer. Buffer ice was added whenever necessary to keep the temperature down, as this faciliated cutting. As they were cut, the slices were transferred with a fine brush onto a small square of filter paper held in the bath with a pair of forceps, and this was placed in a petri dish lined with a circle of filter paper moistened with buffer with 0.1% sodium azide to reduce bacterial growth during storage.

Cells were filled in the caudate nucleus, as the striosomes tend to be better here than in the putamen. The protocol was the same as used in the ferret slices. Cells were filled with 8% lucifer yellow in distilled water, using seven-second long negative current pulses. Cells could be filled on both sides of each slice. After a maximum number of cells had been filled the slice was post-fixed in 4% paraformaldehyde in 0.1m phosphate buffer for at least half an hour (not more than this if immunohistochemistry was to be attempted) and sunk in 20% sucrose in 0.1M phosphate buffer. Forty micron

thick sections were cut on a freezing microtome, either placing the slice on a square of filter paper on top of the ice platform, or sliding the slice from a blade onto the ice platform.

Striosome labelling

After a wash in buffer/saline the slices were incubated in butyrylcholinesterase reagent (Graybiel and Ragsdale, 1978) modified from the Jensen-Blackstad acetylcholinesterase stain;

for 100ml, dissolve in 100ml distilled water:

1,5-bis(4-allyldimethylammoniumphenyl)-pentan-3-one dibromide	0.069g
butyrylthiocholine iodide	0.057g
glycine	0.075g
copper sulphate	0.05g
sodium acetate	0.41g

drops of glacial acetic acid to pH5

The sections were incubated until a white precipitate formed (6-8 hours); it was important to avoid overincubation as this resulted in a crystalline deposit after the DAB reaction. They were then reacted in 10% ferricyanide in distilled water, until the white precipitate turned red-brown. The reaction was stopped in distilled water, and the sections rinsed in 0.9% saline.

Labelling of lucifer yellow

Immunochemistry was performed for the lucifer yellow. The buffer for all the stages and washes was 0.1M phosphate buffer. (Tris buffer was found to remove the BChE label - both precipitate and reaction product). The sections were washed for five minutes in 10% methanol/3% hydrogen peroxide to remove endogenous peroxidase. After rinsing at least three times they were incubated in 1:30 normal goat serum for 30 minutes, which was made up in 0.2% Triton X in phosphate buffer. After another thorough wash they were incubated overnight in the primary antibody. This was optimal at a dilution of 1:16-32 thousand, at which concentration the background labelling was kept to minimum. Also in this solution was 1% normal goat serum (NGS), 1% normal monkey serum (NMS), and 0.01% of thimerosal to inhibit bacterial growth.

The next day the sections were rinsed and incubated in secondary antibody, biotinylated goat anti-rabbit (Vector) 1:500 with 1% NGS, 1% NMS for one hour. They were then washed and incubated in ABC solution (avidin-biotin-peroxidase complex: Vector) (6ul/ml) with the normal sera as before, for another hour. The sections were developed in DAB 0.06% (dissolved initially in distilled water), with 0.003% hydrogen peroxide in 0.1M phosphate buffer. The DAB complexed to label the lucifer yellow-filled cells with a dense precipitate, and also intensified the BChE striosomal stain. The cells were clearly

distinguishable from the BChE-dense striosomes.

The sections were rinsed several times in buffer and then mounted out of 0.5% gelatin in 50% ethanol/50% water onto gelatin-subbed slides. They were mounted in order of cutting, as judged from the location of the lucifer yellow-filled cell bodies and from the tissue landmarks. It was important for the slice to be oriented the same way as when the cells were filled, so that cut dendrites could be followed through consecutive sections and a complete picture of the cells be drawn.

Immunohistochemistry for met-enkephalin- and calcium binding protein-like immunoreactivity were also tried, with inconsistent results. The superficial sections of the slice never showed good staining. If the antibodies to lucifer yellow and to met-enkephalin or CaBP were mixed together at concentrations of 1:32K and 1:1000 respectively, LY-labelled cells could always be found, and striosomes could sometimes be found, however the results were not consistent enough to be used for anaylsis.

Cells were drawn as described in Chapter 3, by using the Eutectics neuron tracing system using visible spectrum light; striosomes could also be drawn at low power. Approximately 200 cells were good fills from 9 slices from 3 animals; of these 53 cells were drawn, 45 from

coronal slices and eight from one sagittal slice (table 4.1).

The dendrites of each cell could be followed through several serial sections (figure 4.1). After all the cut dendrites from each cell had been drawn, each drawing was rotated so that it was aligned correctly, and the cut ends were connected ("merged") to make the cell as complete as possible. Vector diagrams were obtained from each merged cell in order to analyse the orientation of cell with respect to the caudate-putamen. The total length of dendrites in each 15 degree segment in the plane of section is represented by a line in that direction (figure 4.9). In some cases it was necessary to transform the vector data so that the directions relative to the caudate nucleus corresponded appropriately, e.g. when one slice was mounted mirror-image to the rest.

Values were pooled for all cells to be included in each group, and the mean value and standard deviation for each 15^{o} segment was calculated. To compare octants, each three consecutive values were pooled, to get values for $0\text{-}45^{o}$, $45\text{-}90^{o}$ etc., and tested by ANOVA against the values for the other seven octants, in a manner similar to that for the ferret cell data. Similarly the quadrants were compared, each against the other three. Complementary pairs of quadrants ie $0\text{-}90^{o}$ and $180\text{-}270^{o}$ v. $90\text{-}180^{o}$ and $270\text{-}360^{o}$ were compared for each group.

The data was analysed for all cells in coronal slices (n=45) and in various different subgroups. In order to see if cells which were not near striosomes, which did not have dendrites which obviously avoided or were otherwise influenced by the border, this group was described separately (n=13), and compared with the remaining group of cells near striosomes (n=32). Because of the restrictions of the method, it was inevitable that the dendrites projecting towards the superficial surface of the slice would be underrepresented. A group of cells (n=12) which had cell bodies situated somewhat more deeply than the rest were analysed, to see if they were markedly more oriented than those more superficial.

The data were collected from several different slices (unlike the ferret data), and although every effort was made to align them as accurately as possible, it was likely that there would be some rotational error between slices. A group of 18 cells were collected from slice mw6/17, and these were tested separately to see if they were oriented more strongly than the sample as a whole.

The eight cells from one sagittal slice were described separately from the cells in coronal sections.

Results

Description of cells

A wide variety of patterns of dendritic behaviour of the 54 medium spiny neurons were seen (see tables 4.1 and 4.2). Thirty-one cells had their cell bodies located in the BChE-pale matrix, 10 were located inside striosomes, 3 were in border zones, and the rest were not classifiable, due to the border being unclear. All cells drawn were medium-sized spiny neurons. Some cells clearly crossed borders, either being situated on the border and sending dendrites into both striosome and matrix eg mw6/14 cell 1, mw8/6 cell 2 (figures 4.2(a),(b)) or having the cell body situated within a striosome and sending most dendrites out into the matrix e.g. mw6/12 cell 1 (figures 4.2(a),(b),(c)). Another variation was the cell body situated in the middle of a small area of BChE-rich tissue, sending its dendrites radially out into the matrix e.g. mw6/14 cell 5, mw6/17 cell 3 (figure 4.2(a),(e)).

Some neurons clearly avoided crossing striosome/matrix borders, eg matrix cells mw6/17 cells a and c, striosome cell mw8/6 cell 5 (figures 4.3, 4.4). Sometimes a "recurved dendrite" was seen, as identified by Kawaguchi and co-workers (1989), e.g. mw6/12 cell 4 (figure 4.5). Recurved dendrites were also observed in relation to blood vessels - mw6/14 cell 7, mw6/17 cell 4 (figure 4.5).

In other cases dendrites from matrix cells penetrated striosomes by at least 50um, eg mw6/14 cell 2, mw8/1 cell 1 (figure 4.6), or sent just the distal tips of their dendrites over the border eg mw8/6 cell 3, mw9/22a cell 1 (figure 4.7). As might be expected, dendrites were seen to lie along striosome borders eg mw6/17 cells 6 and 8 (figure 4.8), although this did not necessarily mean that they would not send dendrites also across the border eg mw6/17 cell 5 (figure 4.8).

As the striosomes are defined by histochemical stains which stain various elements of the neuropil, one would expect that the orientation dendritic trees of striatal cells would in some way reflect this. Cells which bore a direct relationship to a striosome often had a striking directionality as shown by the vector diagram eg mw6/17 cell c (figure 4.9), however this feature was also seen in matrix cells which were not close to a striosome eg mw6/17 cells 7,h,i (figure 4.9) (see below). One wonders whether this type of cell is related to the "matrisomes" of Graybiel and colleagues (Gimenez-Amaya and Graybiel, 1991; Graybiel *et al.*1991).

Whilst the most complete cells were chosen to be drawn, most cell bodies were located in the most superficial two sections of each slice, and had dendrites extending through 3 or more deeper sections. It was not possible

to fill cells at any greater depth than this. Thus it was inevitable that some part of the dendritic tree would be missing to some extent from all cells. It is, however, unlikely that the missing branches reflect any trend which would not be seen in the remainder of the cell population (see below).

Orientation results

These results are presented in figures 4.10 - 4.14.

Analysis of the orientation of the dendritic fields of medium spiny neurons shows that they tend to have long axis which is oriented dorso-medio-rostral to ventro-latero-caudal, whether they are near to striosomes or not (figure 4.15). This axis is parallel to the long axes of striosomes (figures 4.10, 4.14).

Discussion

The results presented in Chapters 3 and 4 describe the dendritic arborisations of a randomly selected population of medium spiny cells from the caudate nucleus of the squirrel monkey and ferret. It is clear that, although many staining components of these cells are responsible for the histochemically-defined striosomes, in at least 10% of cases dendrites do not respect striosomal borders. This is in contrast to results in the rat (Penny *et al.*1988; Kawaguchi *et al.*1989), in which patch/matrix borders are observed by medium spiny

dendrites. The cells filled in these studies could have been a selected population. Penny and co-workers describe only 10 cells which were in the vicinity of patches. These cells were identified electrophysiologically by their response to stimulation of either cortex or substantia nigra. Cells which were less electrophysiogically stable would not have been filled, although the criterion for recording was a resting membrane potential of only -45 mV.

Kawaguchi and colleagues describe 47 cells which responded to cortical stimulation. It seems less likely that this would be a biased group of medium spiny cells, because there is known to be an extensive input from the cortex to medium spiny cells. They recorded from cells with resting membrane potentials of below -60mV. The selection of these cells could have been biased by the properties of the microelectrode, as the dimensions of the tip could affect which cells were penetrated. This seems a relatively unlikely source of bias as many of the cells filled in the work presented here are apparently of one morphological type - medium size spiny.

This difference in results could be due to a difference between species. There are some differences in striosome staining between species, although in the majority of cases these are not large. At a cellular

level, very similar cell types are seen in most species. There are, however, differences in extra-striatal connectivity, for example, the collateralisation of axonal pathways. It seems possible that the disparity between previous results in the rat, and the results obtained here in ferret and squirrel monkey are due to a species difference, although somewhat unlikely because of other similarities of striatal architecture.

The results presented here agree with work in the cat and the ferret by Bolam and co-workers (1988). They open the way to a more complicated interpretation of the significance of the striosomal system. The afferents of striosomes and matrix are in many cases distinct (see Chapter 3), and the medium spiny neurons of either compartment, identified by retrograde labelling of their cell bodies, project to different targets. The interneurons of the striatum, of different types, are known to cross striosome/matrix borders, but this new information suggests that input to the complementary compartment can directly influence output without the use of interneurons (figure 4.16).

The orientation data indicate that the dendritic fields of medium spiny striatal cells are aligned with the long axes of striosomes, from rostro-dorso-medial to caudo-ventro-lateral. This is perhaps not surprising for cells which are in the vicinity of borders between the two compartments, as the neuropil of these cells is what

constitutes the immunohistochemically-defined striosomes. However, the orientation is just as marked for cells which are not close to borders, suggesting that there is another factor which is important in determining orientation.

Further implications of these results are discussed in Chapter 5.

Table 4.1 Location of cell body and dendrites with respect to BChE-dense striosomes: cells from coronal slices

Cell #	type	cell body	dendrites
mw6/7c1	m sp	unclear	in/out
7c2	m sp	none	in/out into BuChE+ matrix
mw6/12c1	m sp	in	out
12c2	m sp	out	out (not near)
12c3	m sp	out	one dendritic in
12c4	m sp	out	stay out
mw6/14c1	m sp	border	in/out
14c2	m sp	out	in
14c3	m sp	out	out
14c4	m sp	out	out (not near)
14c5	m sp	in	radially out
14c6	m sp	in	stay in
14c7	m sp	out	out (not near)
mw6/17c1	m sp	out	out (not near)
17c2	m sp	out	out (not near)
17c3	m sp	in	radially out
17c4	m sp	out	tip in
17c5	m sp	out	in/out/along border
17c6	m sp	out	out/along border
17c7	m sp	out	out (not near)
17c8	m sp	out	stays out
17c9	m sp	in	stays in
17ca	m sp	out	out
17cb	m sp	out	out
17cc	m sp	out	out
17cd	m sp	out	out
17ce	m sp	out	out (not near)
17cf	m sp	out	out (not near)
17cg	m sp	in	stays in
17ch	m sp	out	out (not near)
17ci	m sp	out	out (not near)
mw8/1c1	m sp	out	1 dendrite in
1c2	m sp	in	out
mw8/3c1	m sp	out	in/out
3c2	m sp	unclear	in/out
mw8/5c1	m sp	unclear	in/out
5c2	m sp	out	out
mw8/6c1	m sp	in	stays in
6c2	m sp	border	in/out
6c3	m sp	in	in/tips out
6c4	m sp	out	out (not near)
6c5	m sp	in	stays in
6c6	m sp	none	out
6c7	m sp	out	out (not near)
6c8	m sp	out	out (not near)

Table 4.2 Location of cell body and dendrites with respect to BChE-dense striosomes: cells from sagittal slices

Cell #	type	cell body	dendrites
mw9/22ac1	m sp	out	tips in
22ac2	m sp	unclear	in
22ac3	m sp	border	in/out into BChE+matrix
22ac4	m sp	in	stays in
22ac5-8	m sp	out	out (not near)

m sp=medium spiny

Figure 4.1(a) Slice MW6/14 section 1; 40μm thick section showing striosomes (dark areas stained for butyrylcholinesterase) and lucifer yellow-filled neurons, labelled with DAB; from top to bottom, cell 5, cell 3, cell 2, cell 1

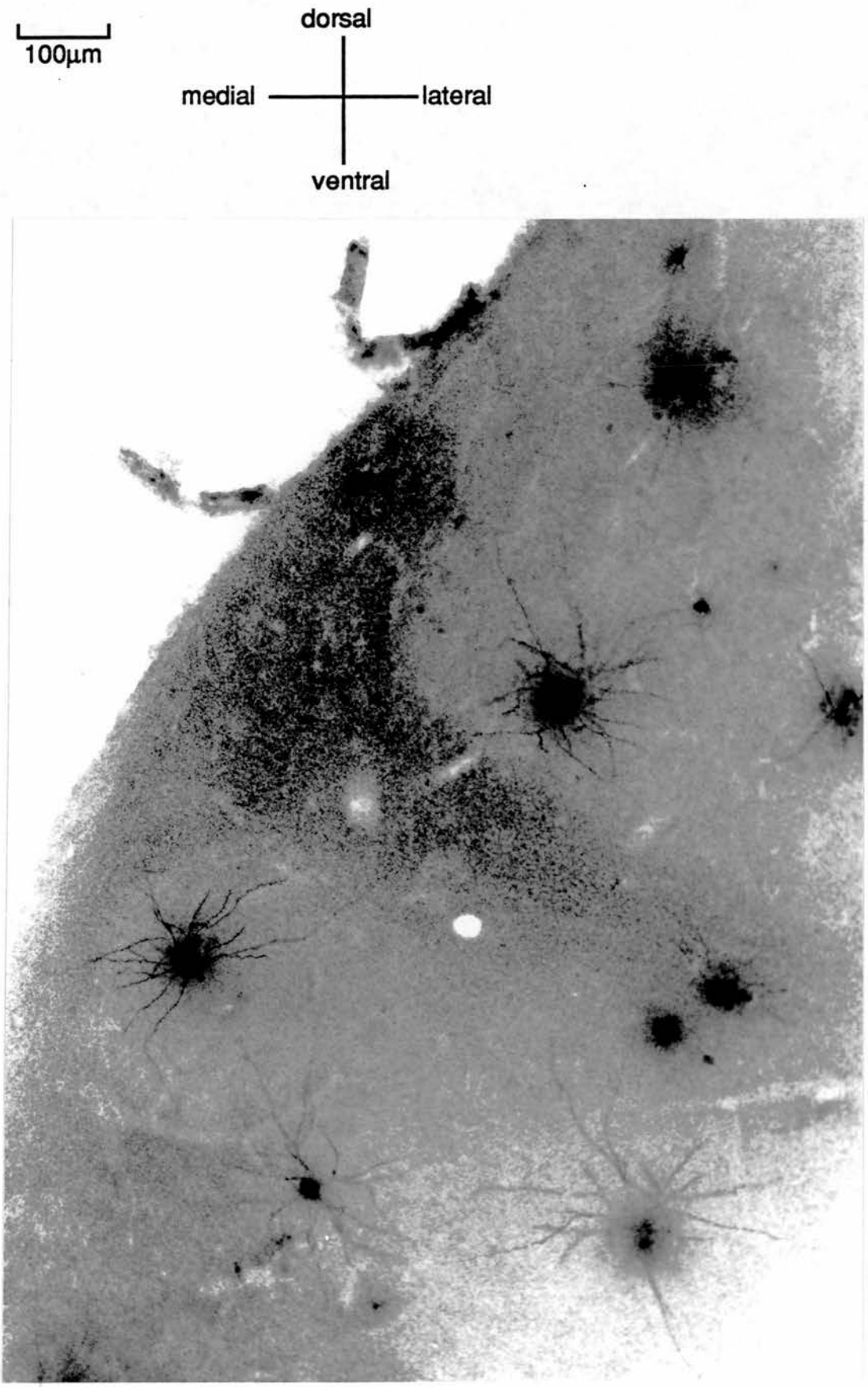


Figure 4.1(b) Section 2, serially adjacent to (a), showing continuations of dendrites

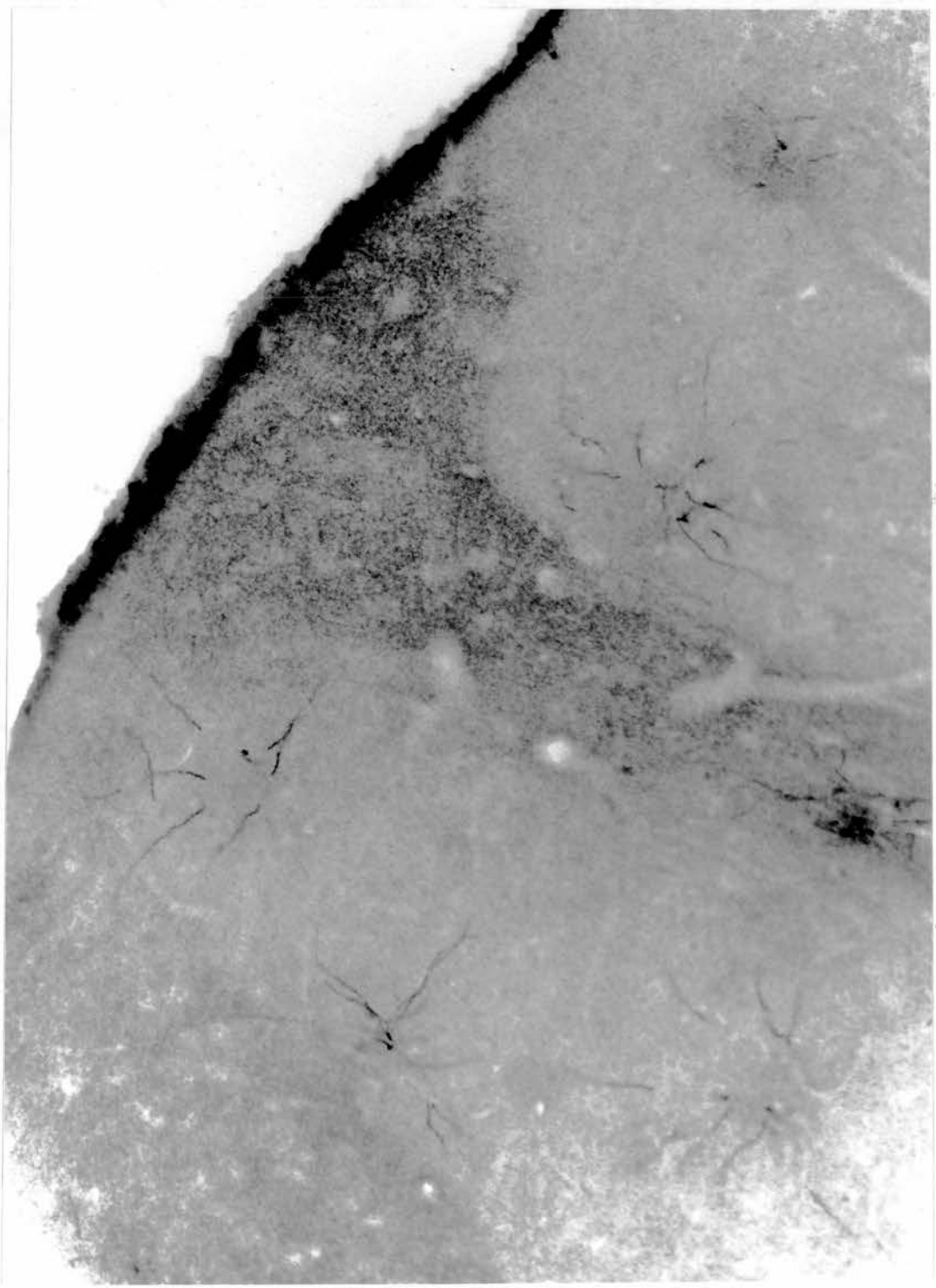
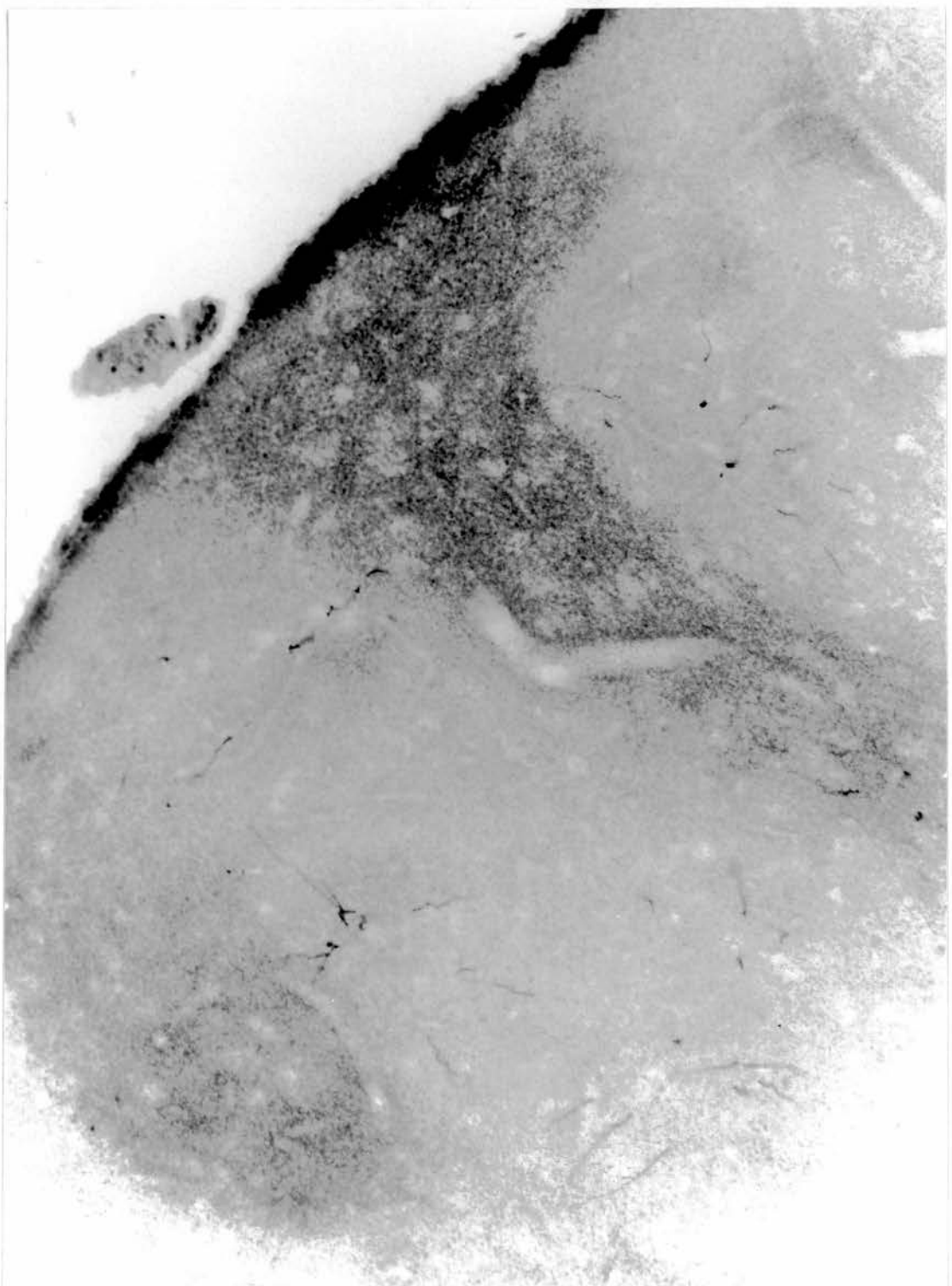
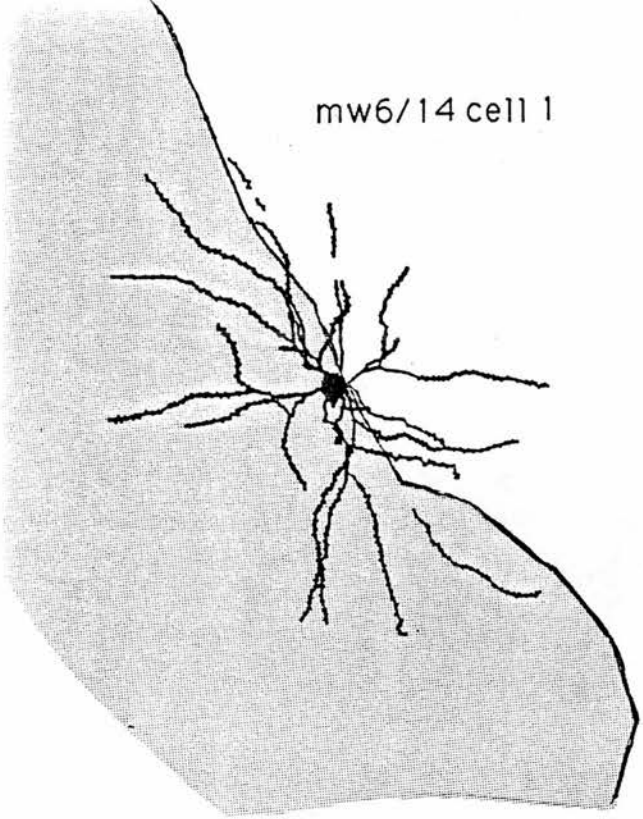
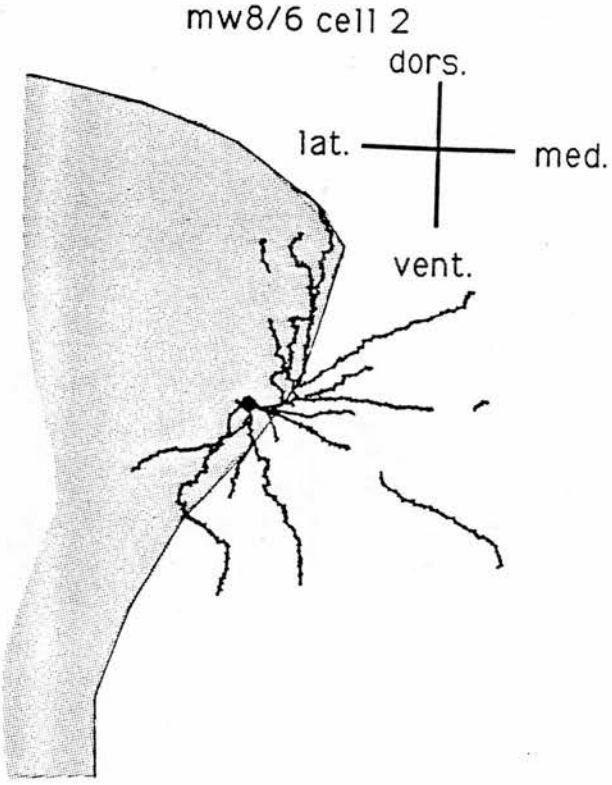
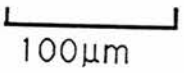


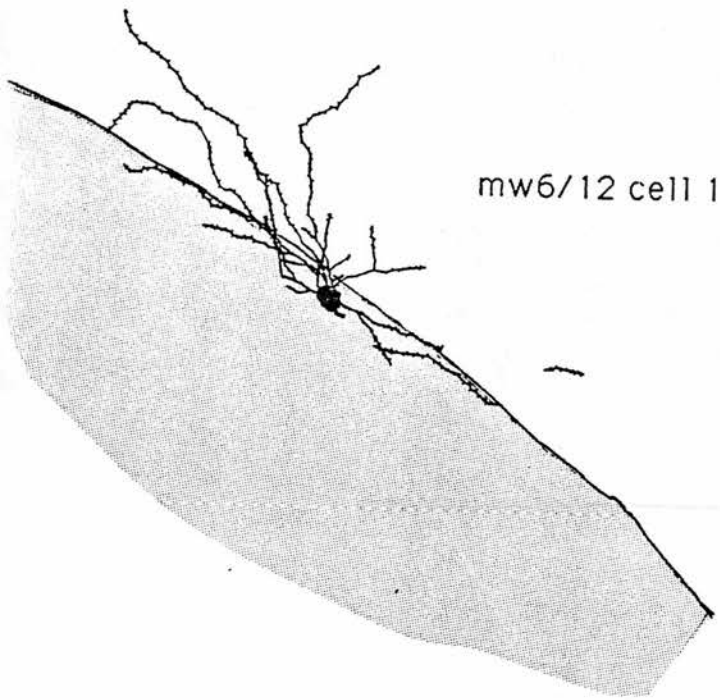
Figure 4.1(c) Section 3, serially adjacent to (b)

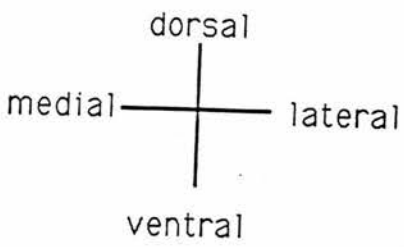


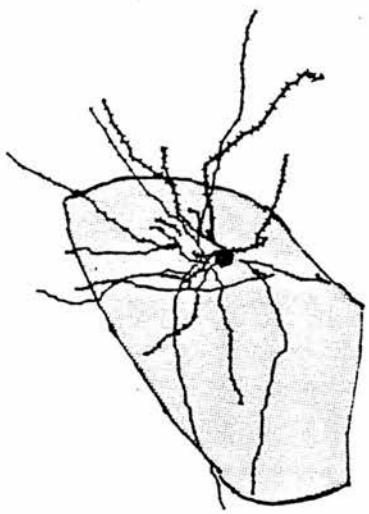

mw6/14 cell 1


mw8/6 cell 2
dors.
lat.
med.
vent.


100µm


mw6/12 cell 1


dorsal
medial
lateral
ventral


mw6/17 cell 3

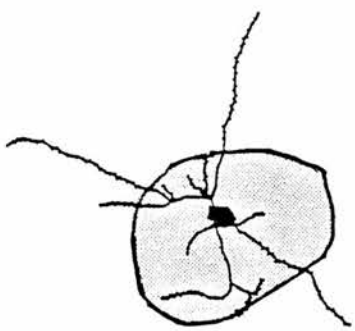

mw6/14 cell 5

Figure 4.2(a) [facing] Cells crossing striosome/matrix borders

(b) mw6/14 cell 1

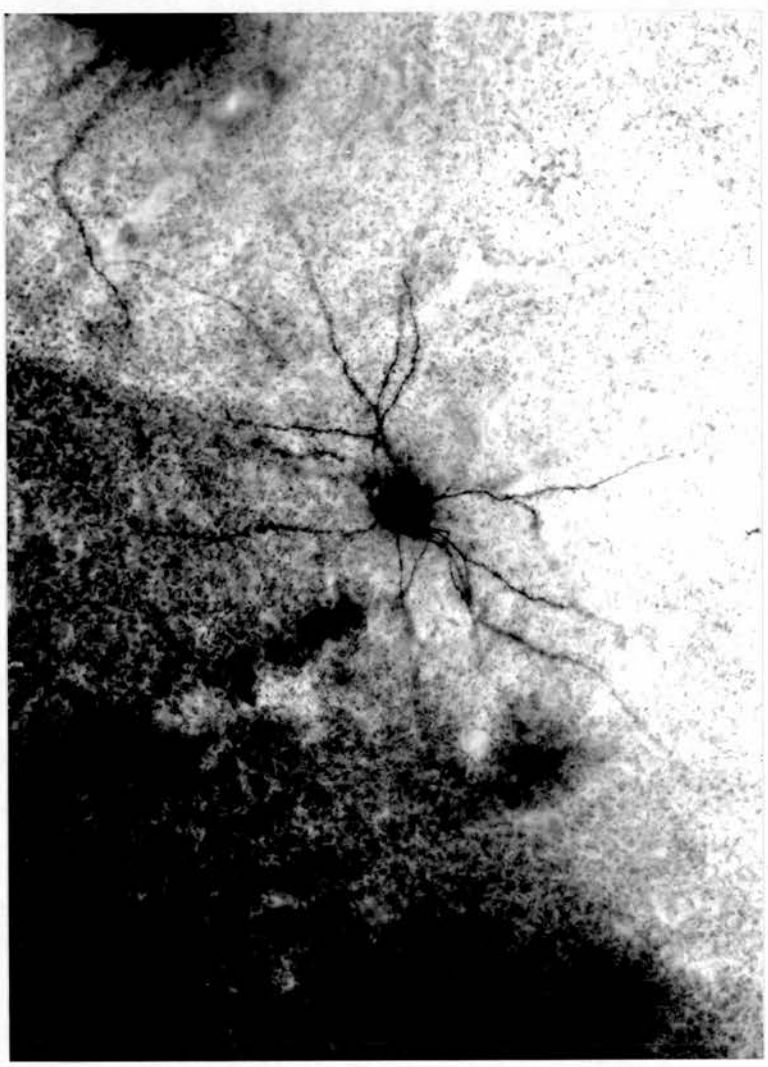
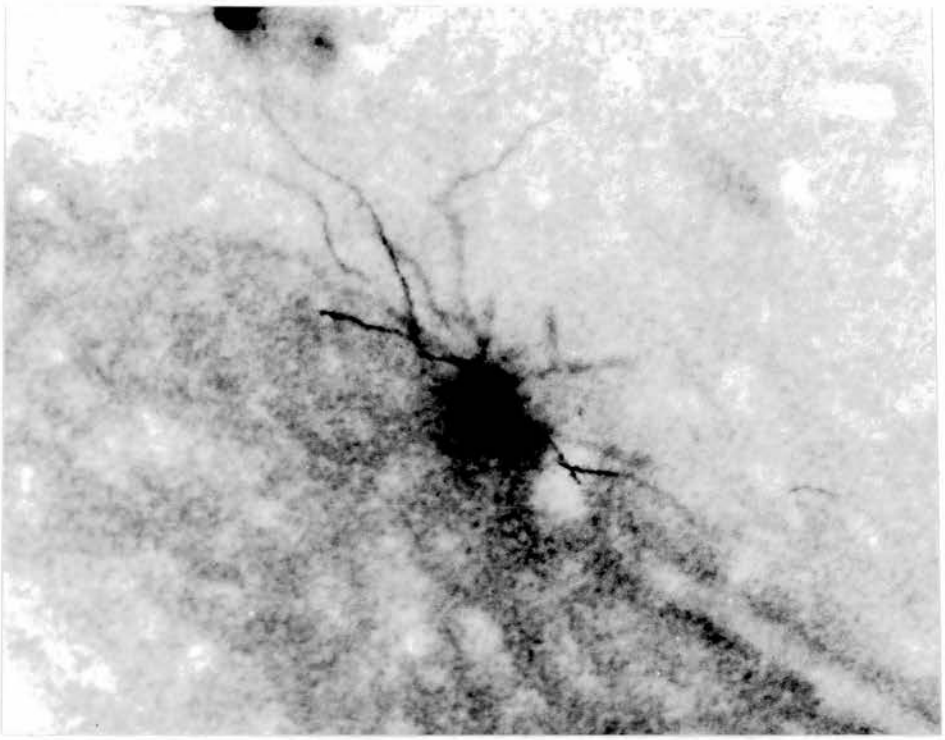


Figure 4.2(c) mw6/12 cell 1 section 1



(d) mw6/12 cell 1 section 2

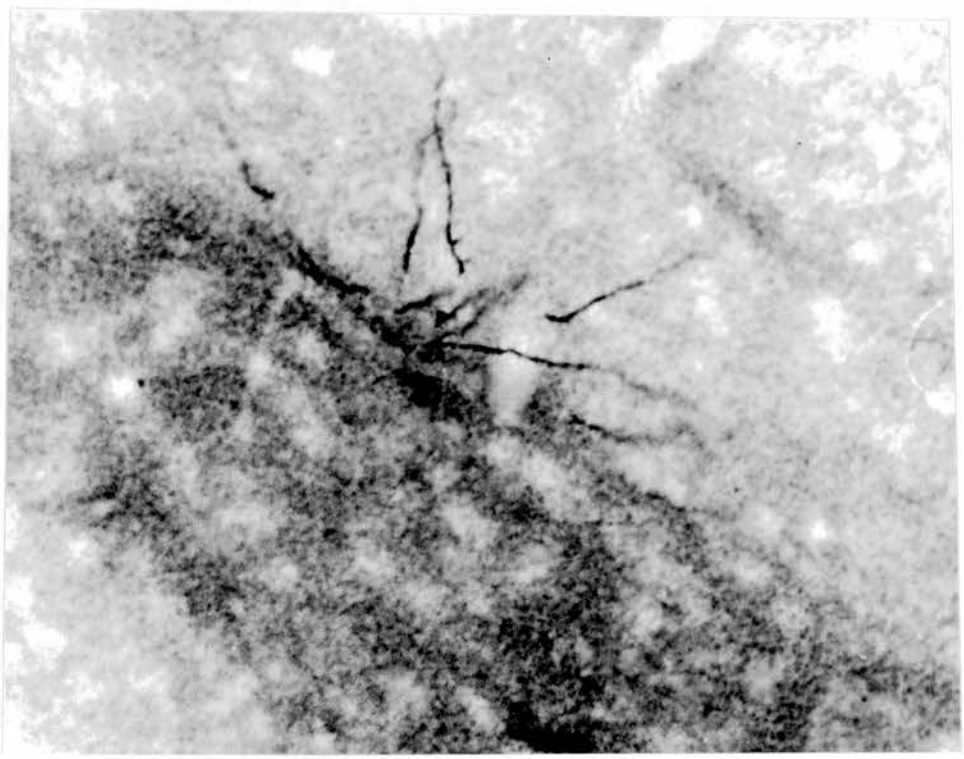


Figure 4.2(e) mw6/17 cell 3

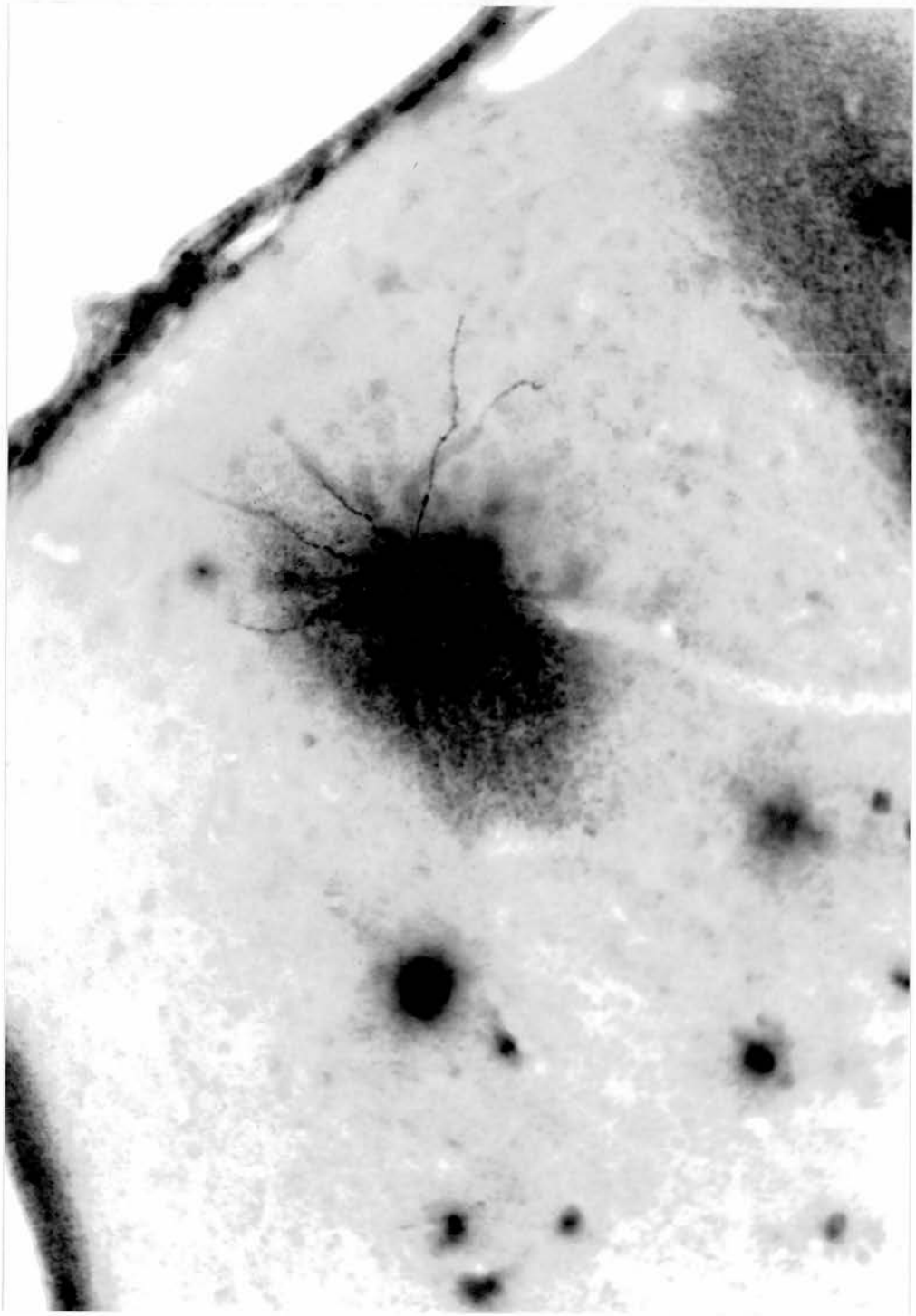
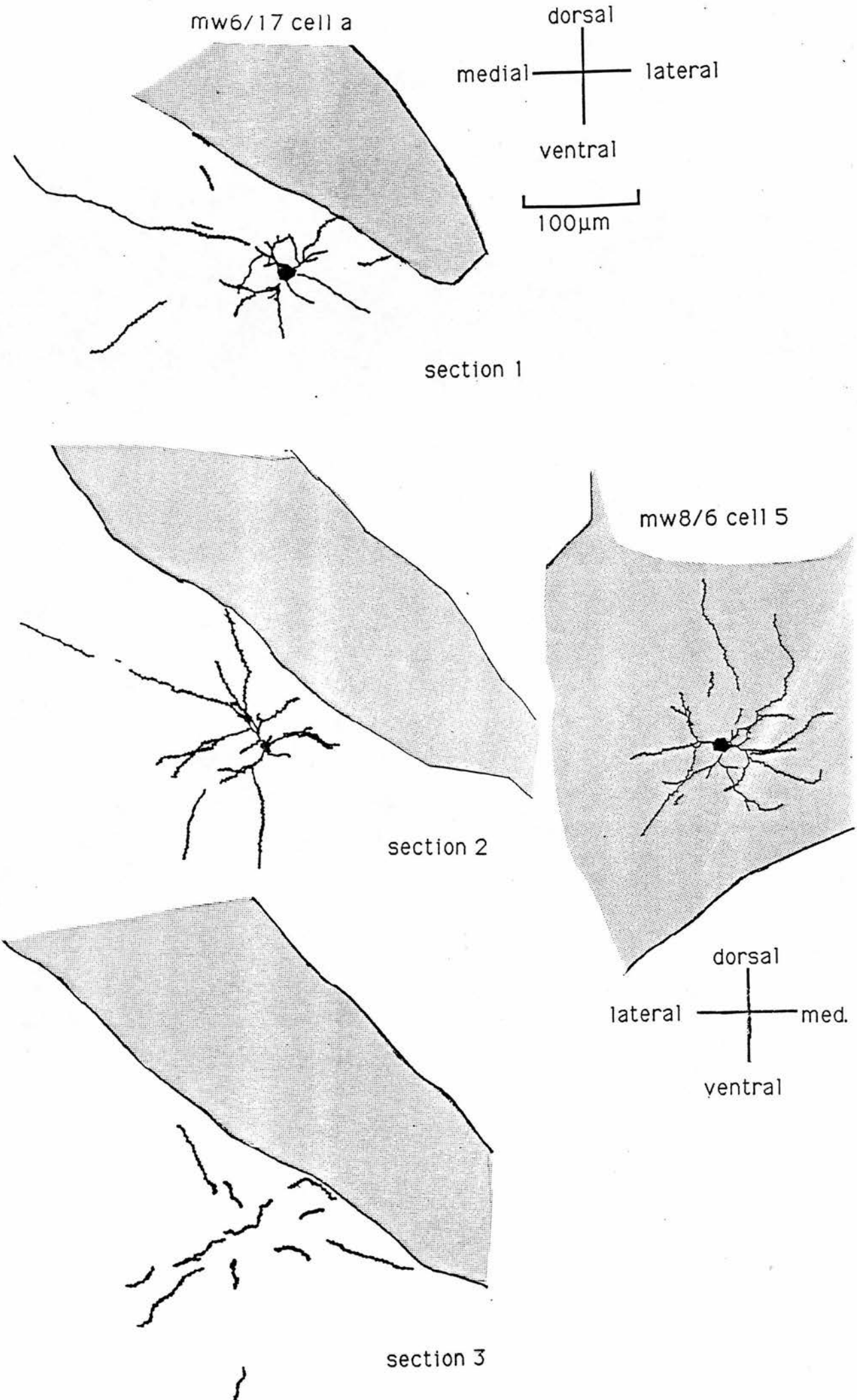


Figure 4.3 Cells not crossing borders. Serially adjacent sections (1-3) shown for mw6/17 cell a


mw6/17 cell a
dorsal
medial
lateral
ventral
100µm
section 1
section 2
mw8/6 cell 5
dorsal
lateral
med.
ventral
section 3

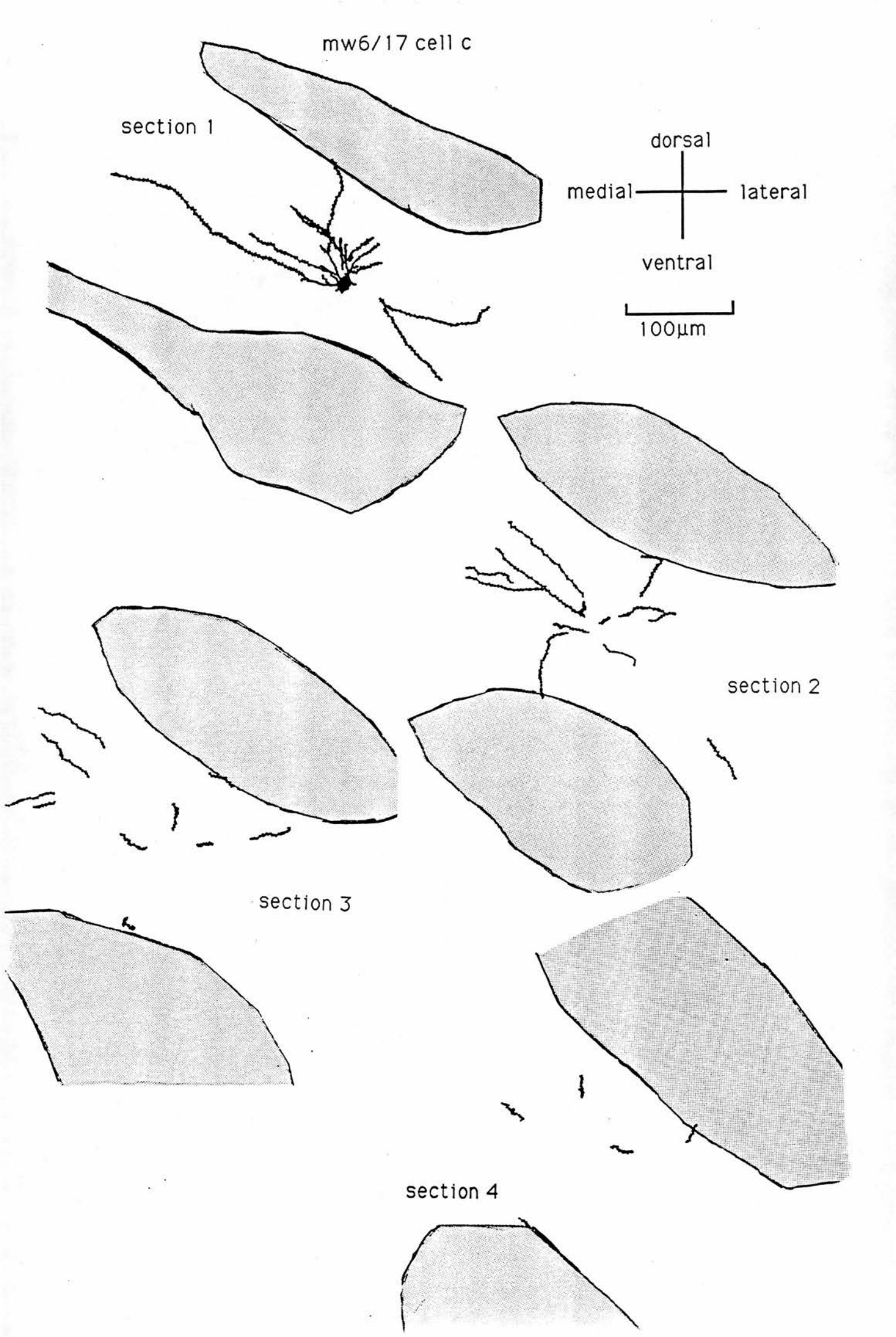
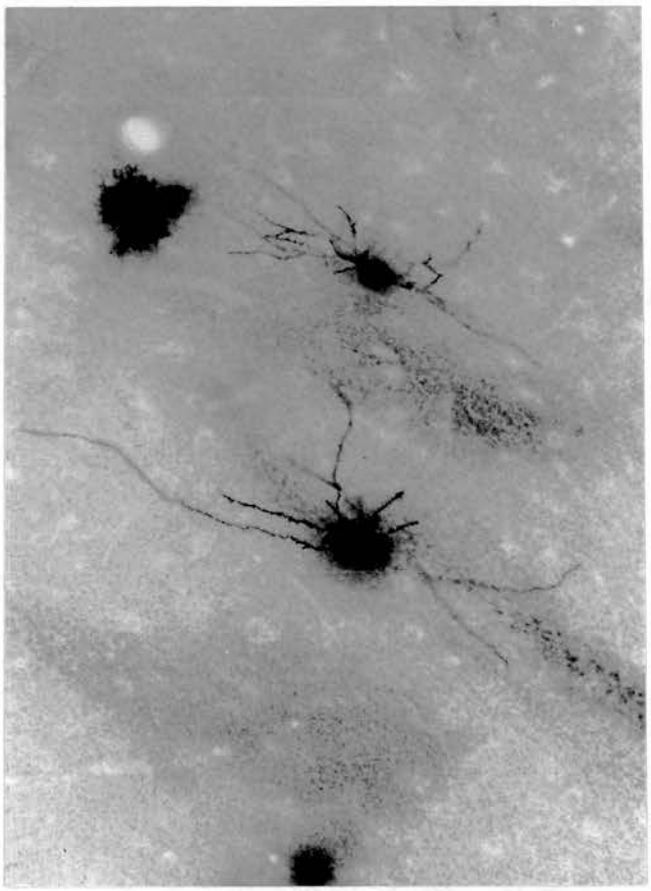

mw6/17 cell c
section 1
dorsal
medial
lateral
ventral
100µm
section 2
section 3
section 4

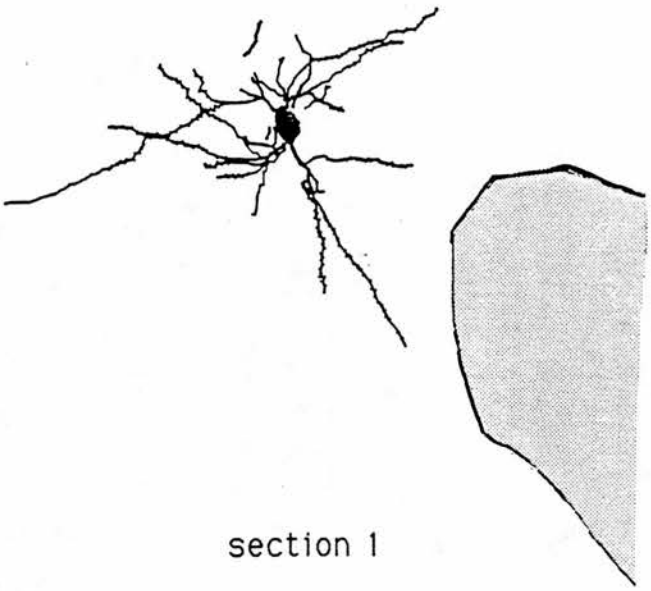
Figure 4.4(a) [facing] cell not crossing borders, serial sections (1-4)

(b) mw6/17 cell c section 1, also mw6/17 cell d, and neurogliaform cell, upper left

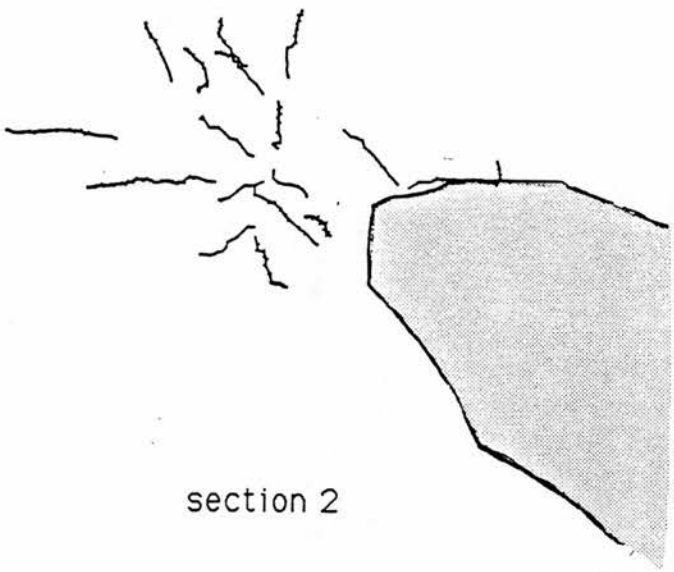


(c) mw6/17 cell (c) section 2

mw6/12 cell 4

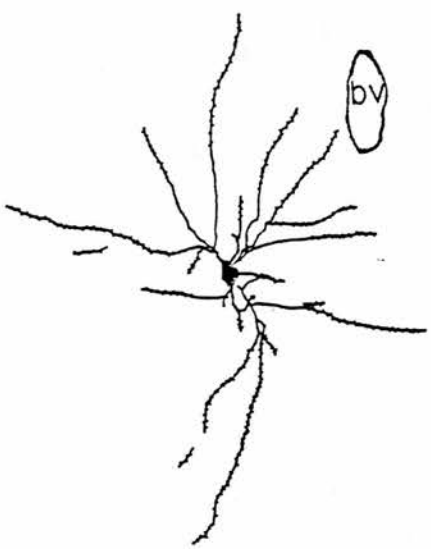


section 1

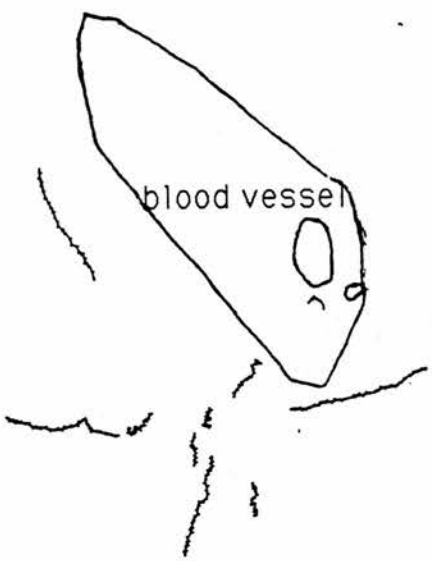


section 2

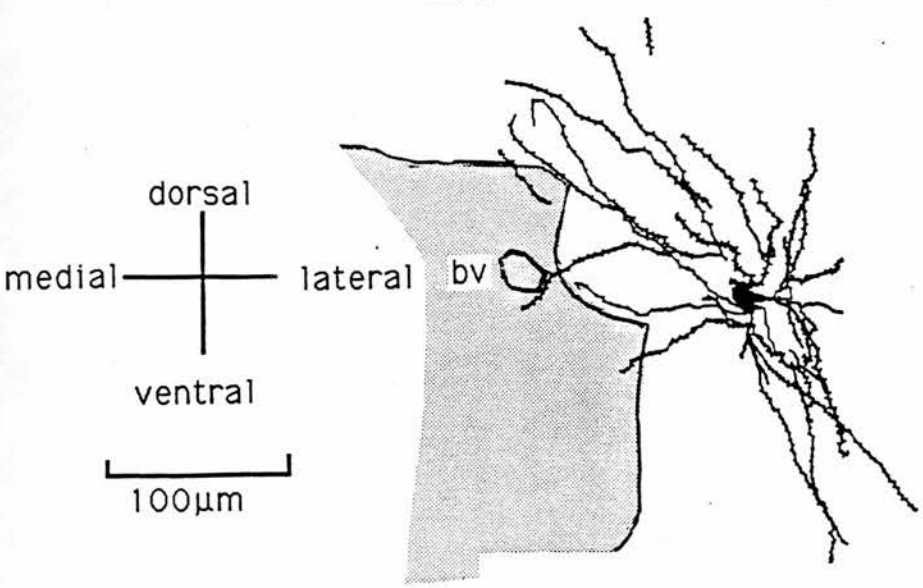
mw6/14 cell 7



section 1



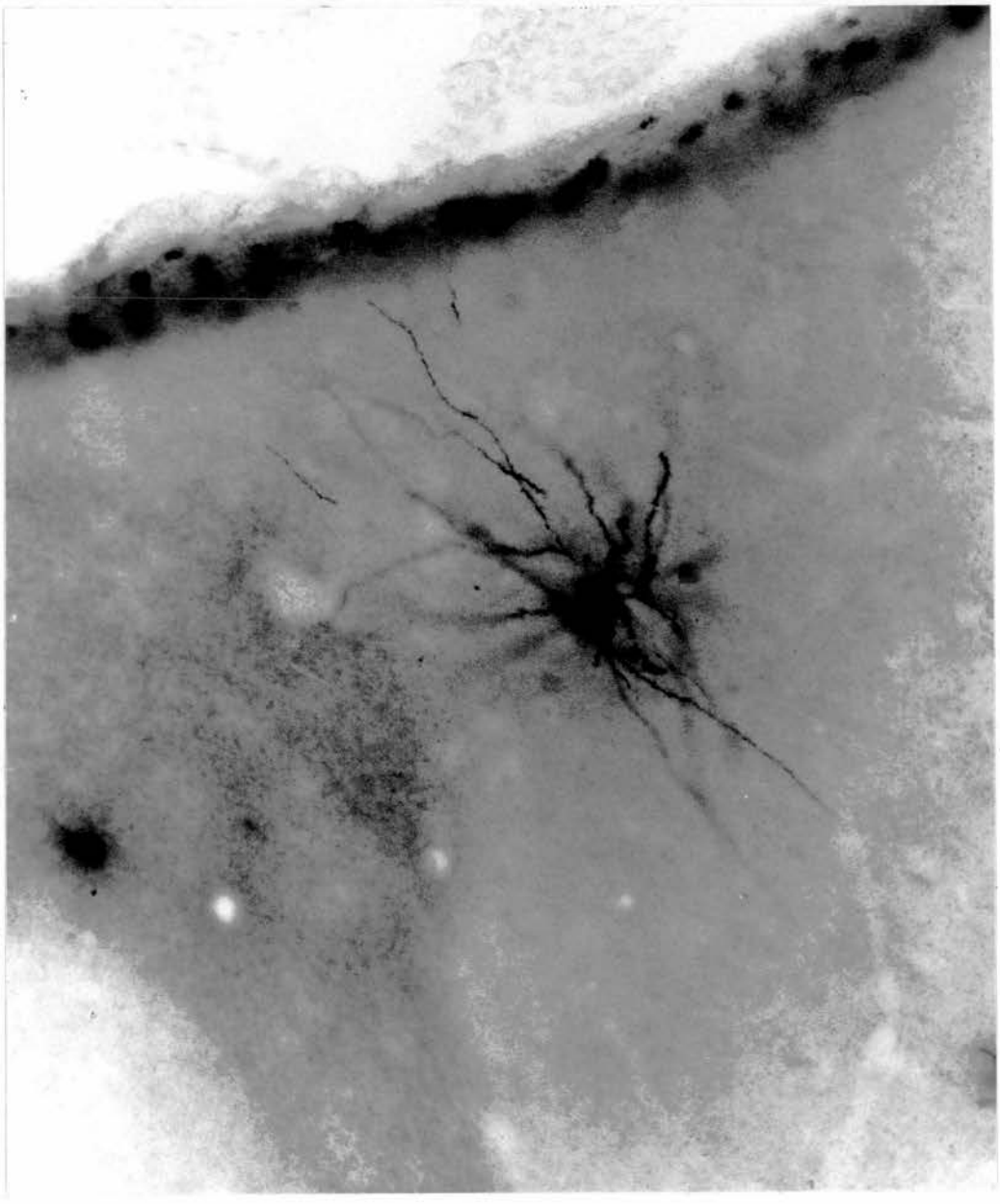
section 2

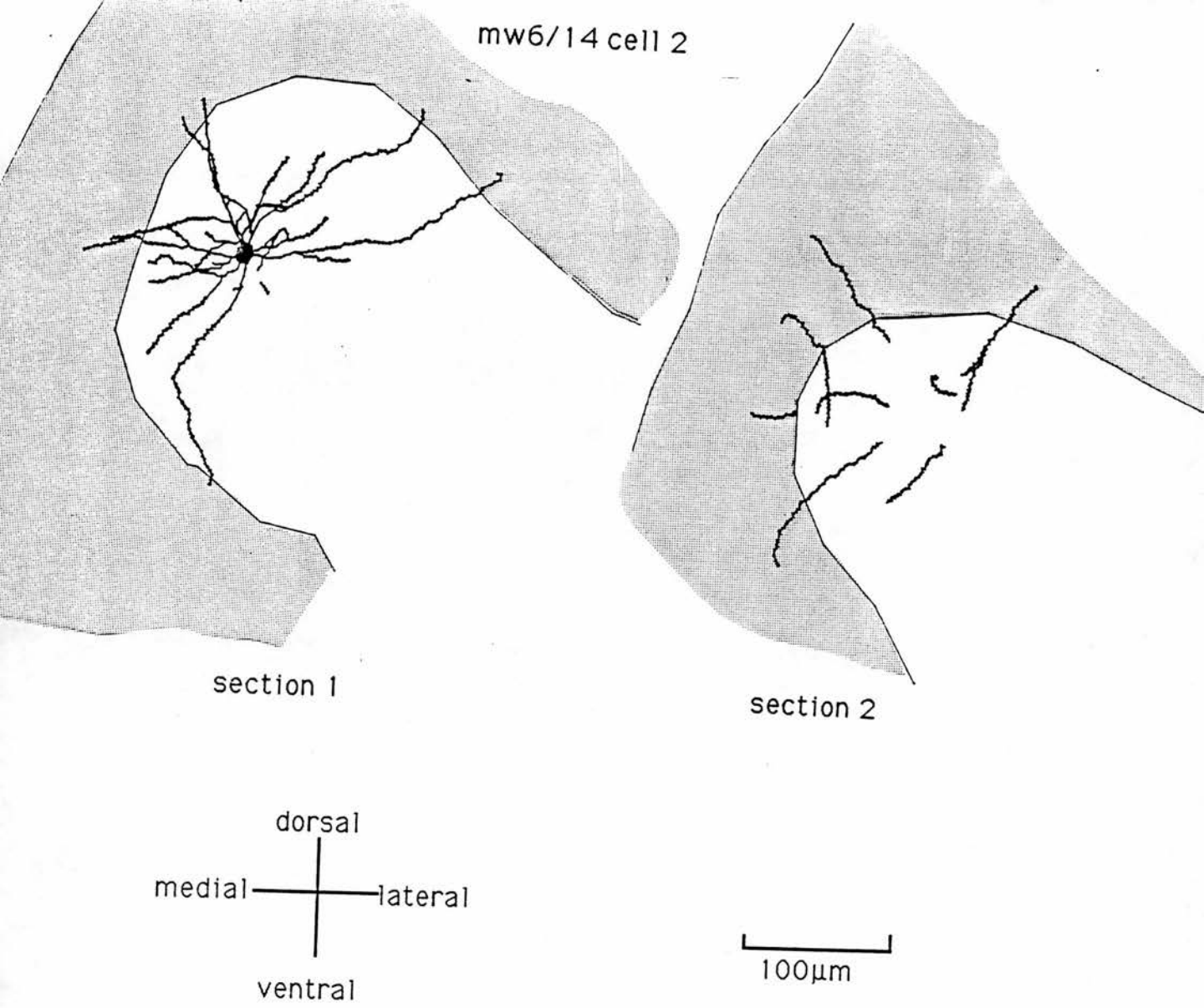


mw6/17 cell 4

Figure 4.5(a) [facing] Recurved ends of dendrites at striosome borders or blood vessels

(b) mw6/17 cell 4




mw6/14 cell 2
section 1
section 2

dorsal
medial
lateral
ventral

100µm

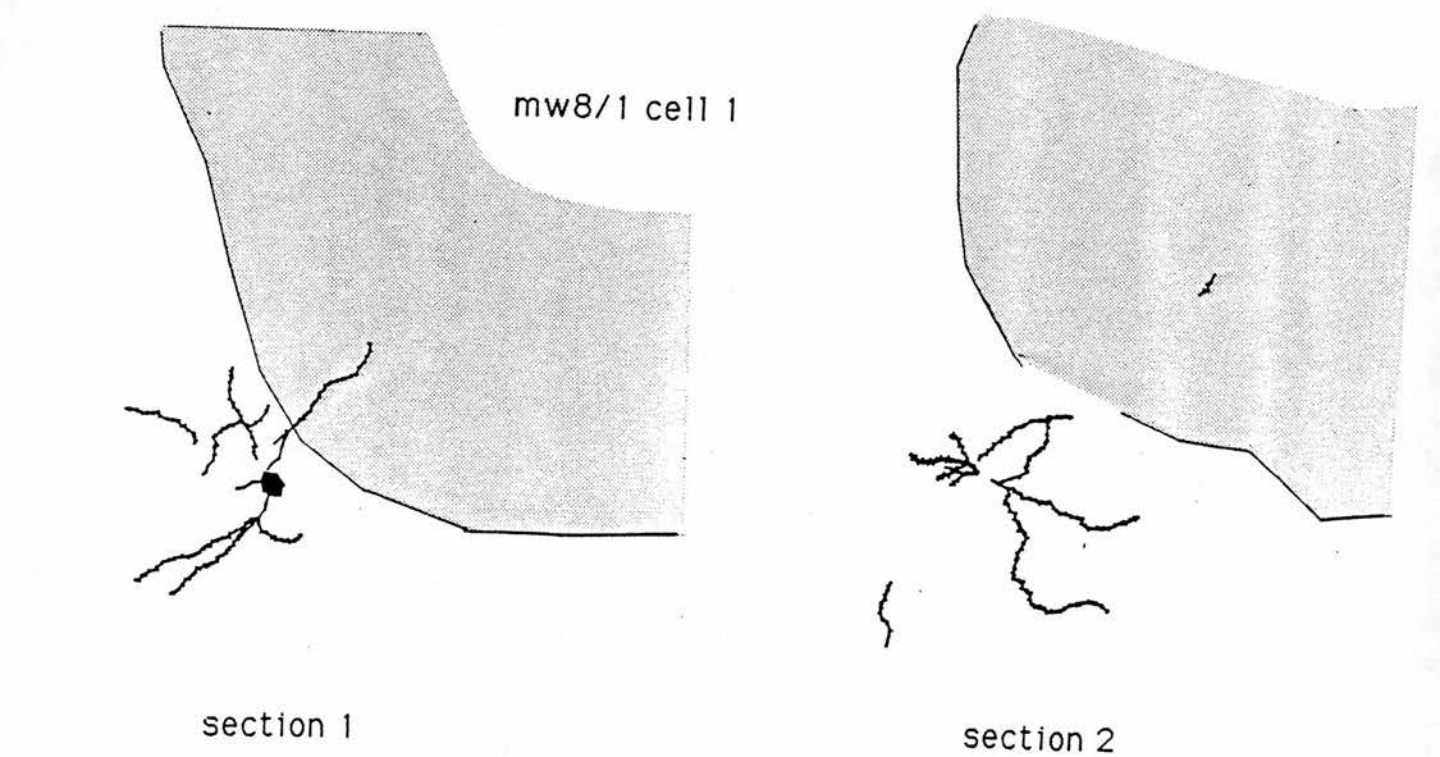

mw8/1 cell 1
section 1
section 2

Figure 4.6 (a) [facing] Distal dendrites entering striosomes, serial sections (1-2)

(b) mw6/14 cell 2

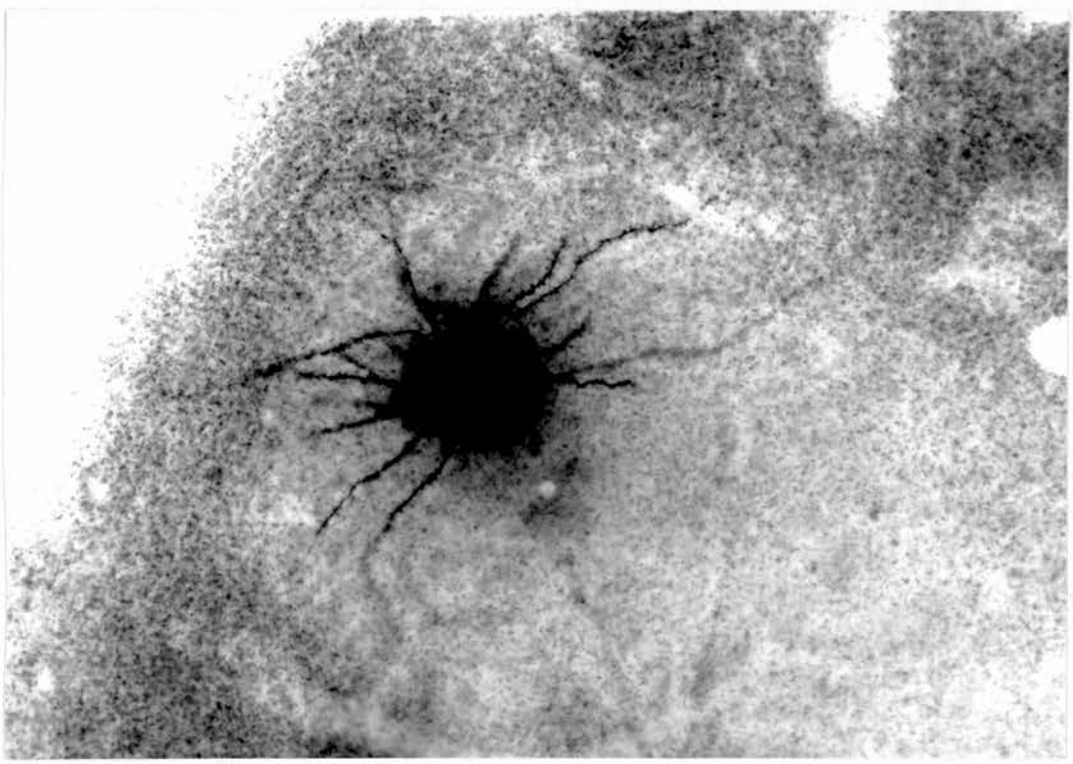
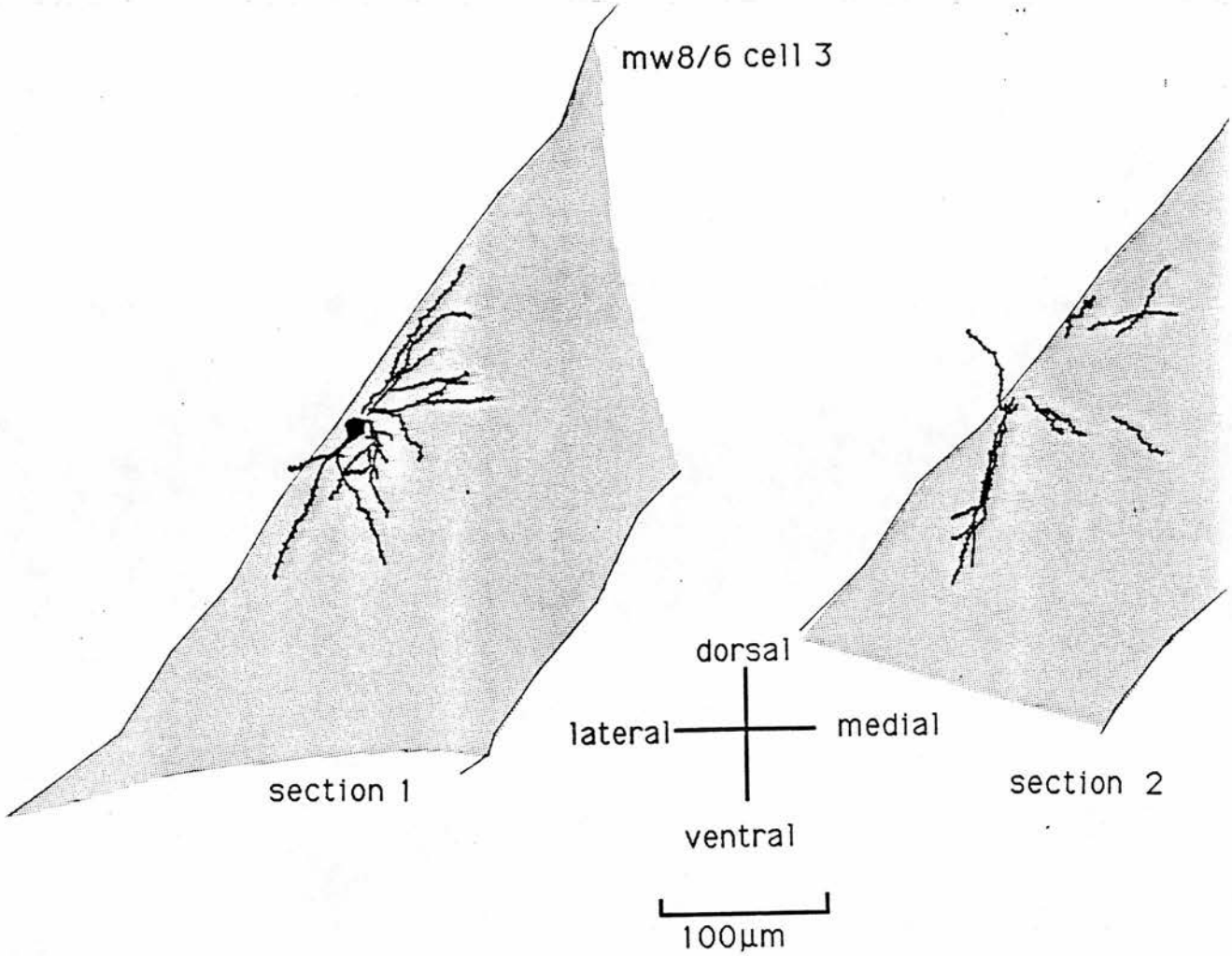
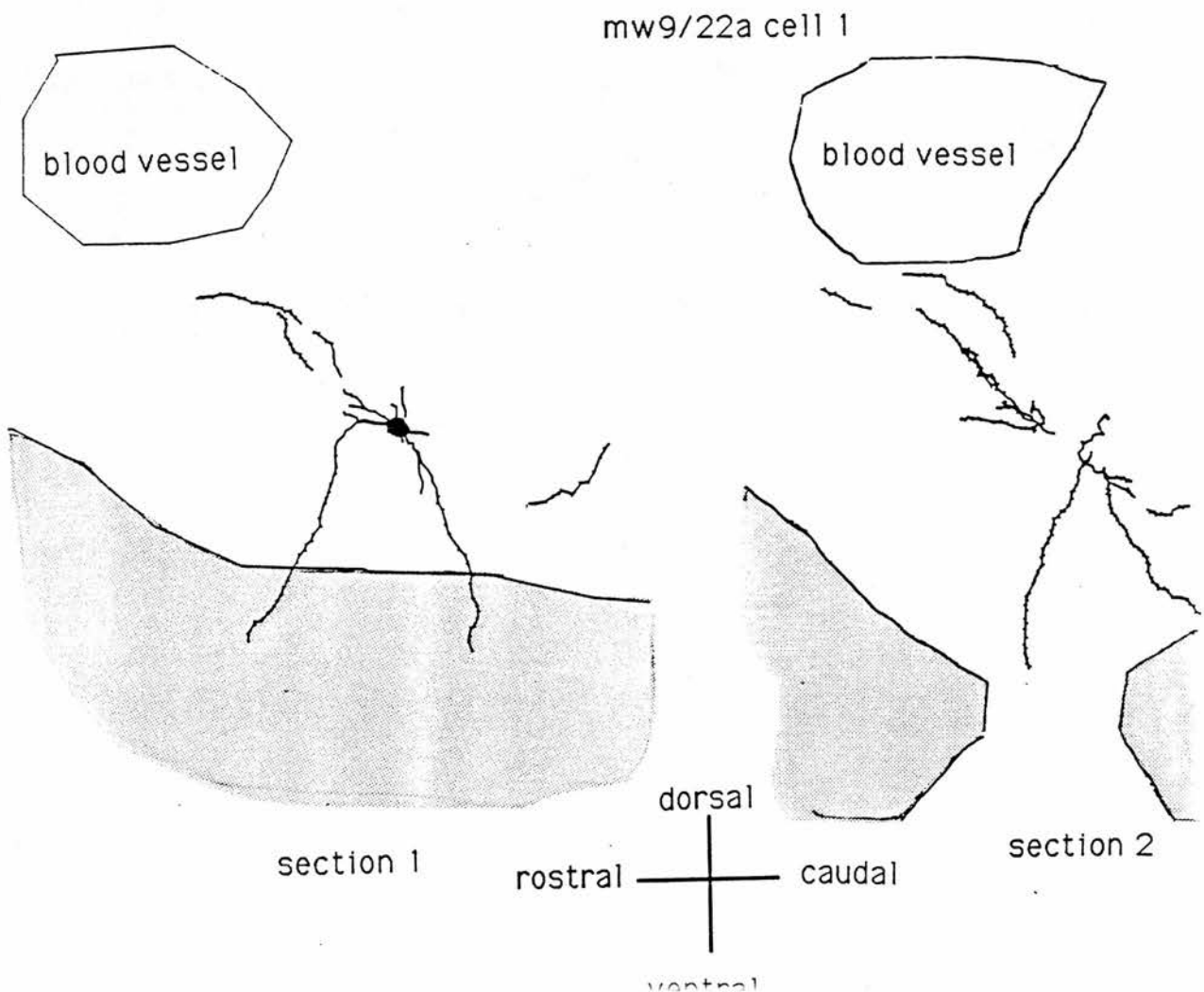


Figure 4.7 Distal dendrites exiting/entering striosomes


mw8/6 cell 3
section 1
section 2
dorsal
lateral
medial
ventral
100µm


mw9/22a cell 1
blood vessel
blood vessel
section 1
section 2
dorsal
rostral
caudal
ventral

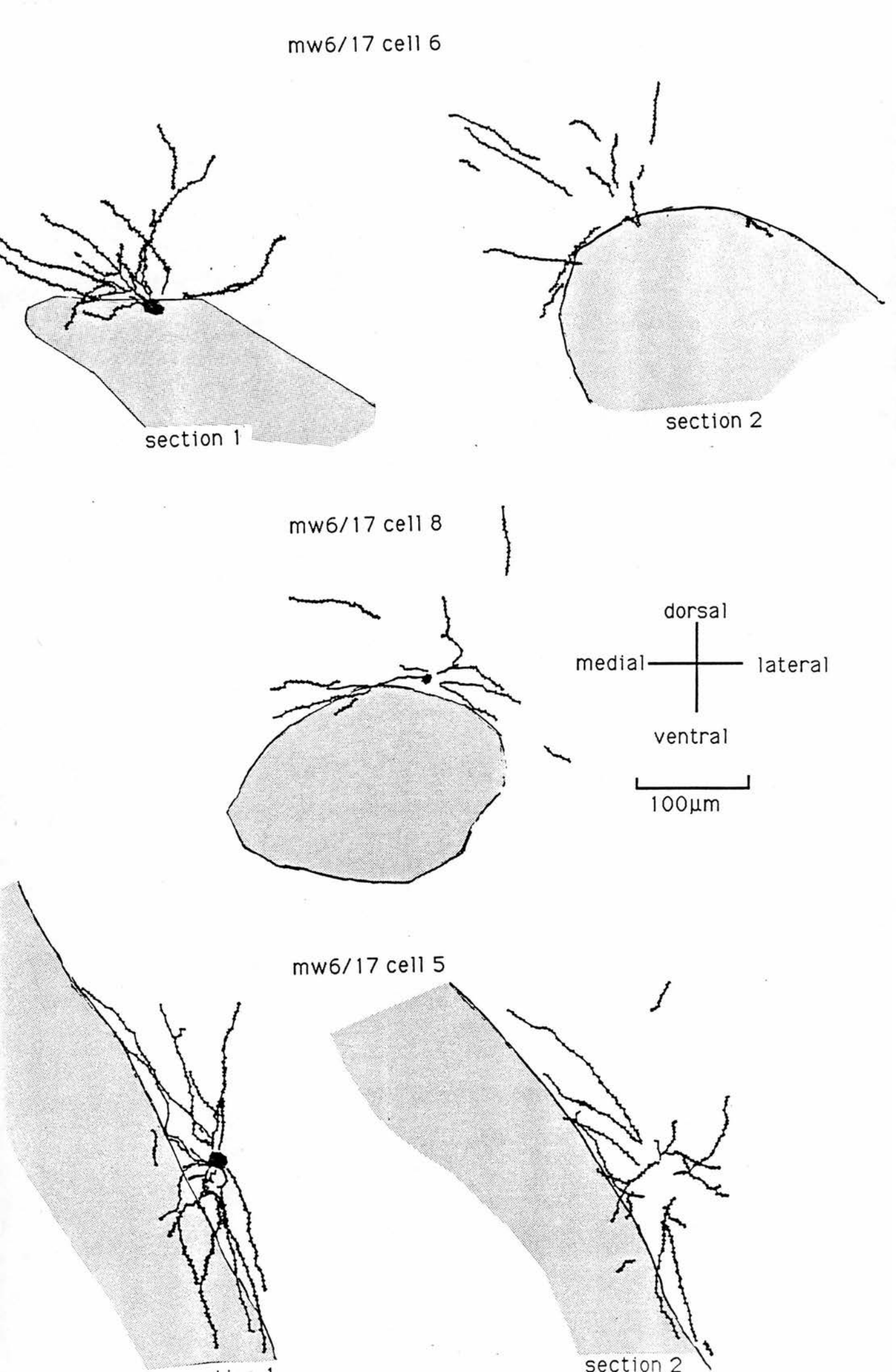

mw6/17 cell 6
section 1
section 2
mw6/17 cell 8
dorsal
medial
lateral
ventral
100µm
mw6/17 cell 5
section 2

Figure 4.8 (a) [facing] Dendrites following borders
(b) mw6/17 cell 5

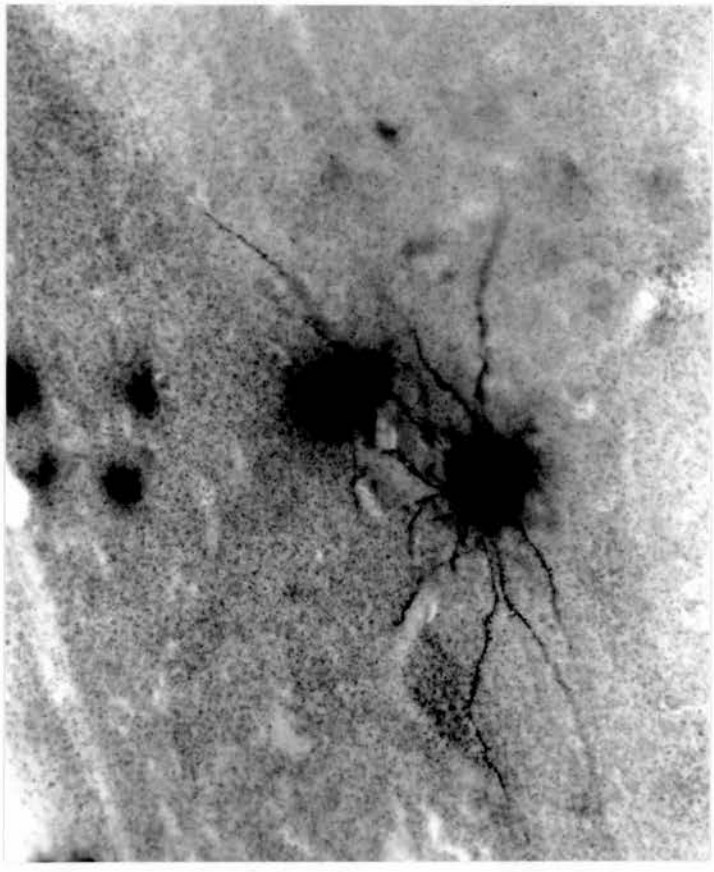


Figure 4.9 Cells with dendrites from all serial sections joined up ("merged"), and their vector diagrams, indicating the amount of dendritic length in each 15^{o} segment surrounding the cell body

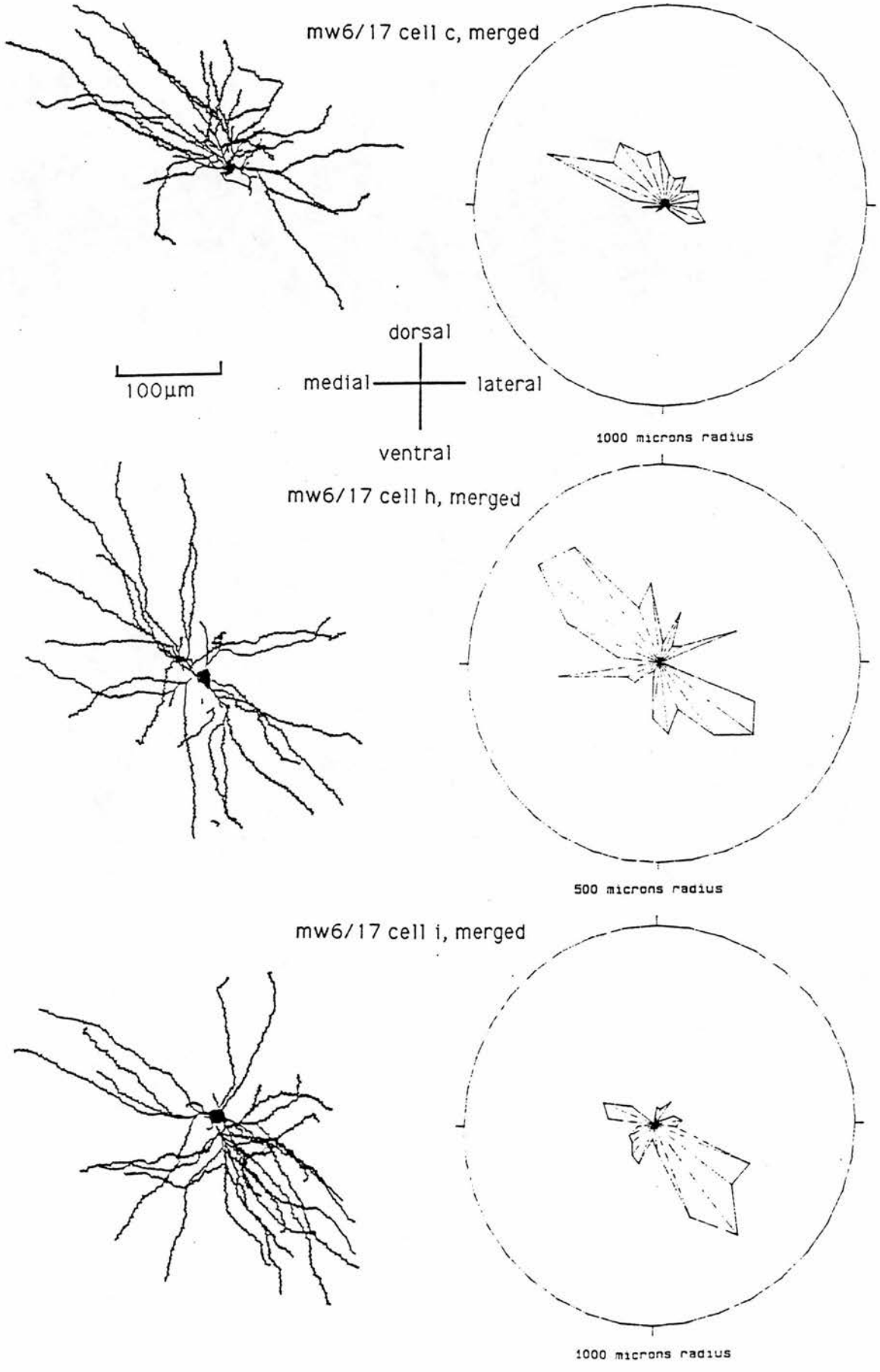

mw6/17 cell c, merged
dorsal
medial
lateral
ventral
100µm
1000 microns radius
mw6/17 cell h, merged
500 microns radius
mw6/17 cell i, merged
1000 microns radius

Figure 4.10(a) <u>All coronal cells (n=45)</u>

The mean value for each 15^{o} segment is given, with a line representing the standard deviation radiating outwards only, to avoid confusion.

The mean values for directional lengths of dendrites were significantly for the medio-dorsomedial octant larger than other directions p=0.0013 by ANOVA.

The dorsomedial quadrant was significantly larger p=0.0002.

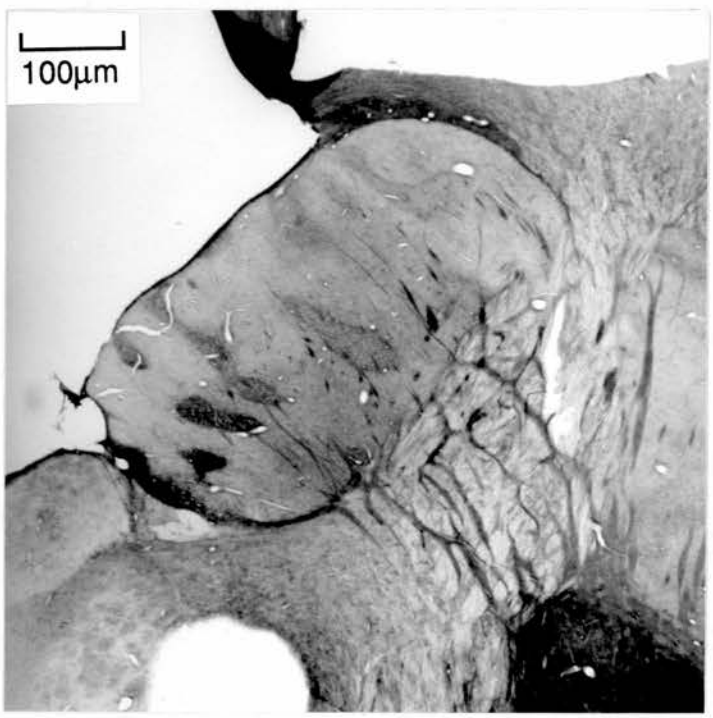
The dorsolateral quadrant was significantly smaller p=0.03.

The ventro-ventromedial octant was significantly smaller at p=0.042.

The dorsomedial and ventrolateral quadrants together were larger than the ventromedial and dorsolateral quadrants p=0.0001.

The dorsomedial quadrant was significantly larger than the ventrolateral quadrant p=0.016.

(b) Coronal section through squirrel monkey brain, stained for butyrylcholinesterase, oriented as diagram on facing page



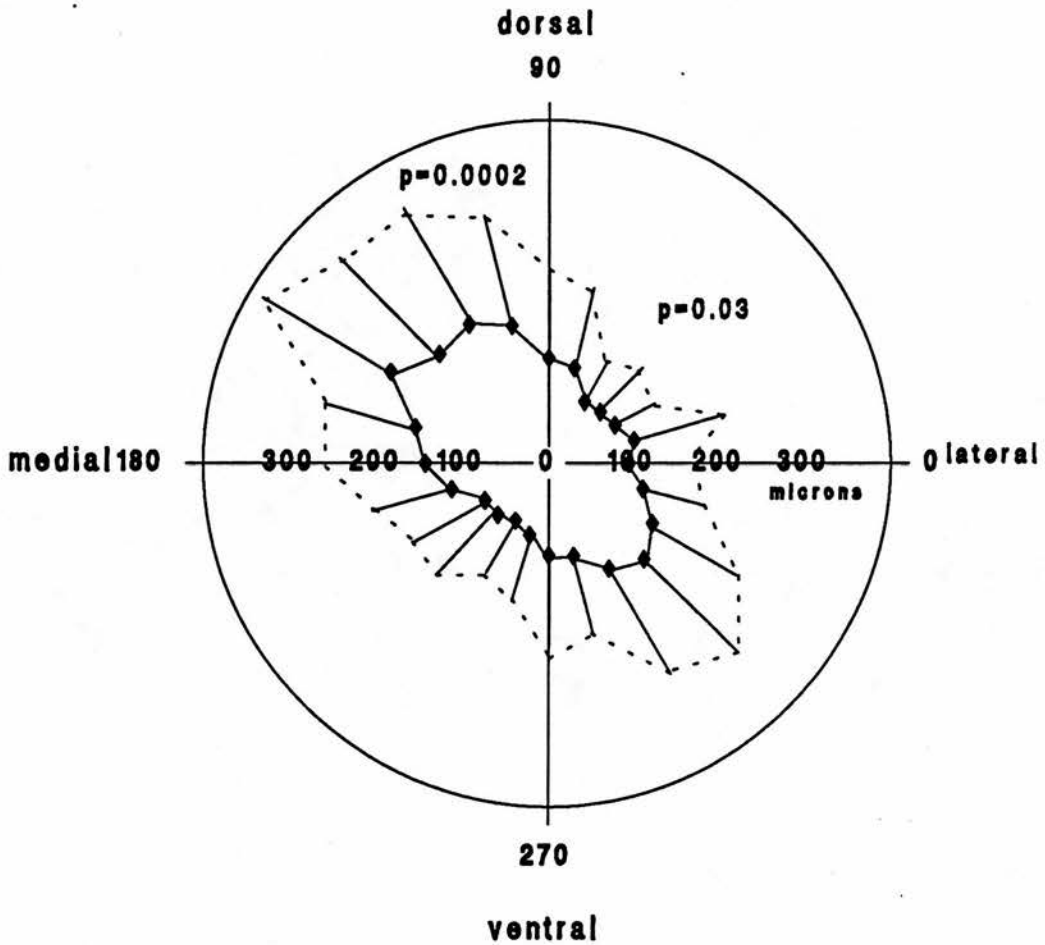

dorsal
90
p=0.0002
p=0.03
medial 180
300
200
100
0
100
200
300
microns
0 lateral
270
ventral

Figure 4.11 Cells not near striosomes (n=13)

The values representing dendritic length projected onto 2 dimensions were 156.6+/-52.8 (mean+/-sd) for cells not near striosomes, compared to those near striosomes 109.1+/-35.2. This difference of 143.5%, was statistically significant by paired t test at p=0.0001. This may indicate that cells near striosomes have shorter dendrites. An alternative explanation would be that they have dendrites oriented more in a rostrocaudal plane. However, if this is true, it was not seen in the cells examined here from the sagittal slice, or in the ferret cells from the horizontal slice.

For the cells not near striosomes, the orientation was marked. The dorsomedial quadrant being significantly larger p=0.008; the dorsolateral quadrant was significantly smaller p=0.003.

The medio-dorsomedial octant was significantly larger at p=0.018.

Again the complementary quadrants were significantly different at p=0.0001. The dorsolateral quadrant was significantly smaller than its opposite quadrant p=0.043.

Cells near striosomes (n=32)

For the cells more closely related to striosomes, the orientation was very marked, with the dosromedial quadrant being larger p=0.0001, and its component octants, the dorso-dorsomedial octant larger at a significance level of 0.047, and the medio-dorsomedial octant strikingly larger at p=0.0007.

The ventromedial quadrant was smaller at p=0.04.

The ventro-ventromedial octant was smaller than the rest at p=0.02 and complementary quadrants were significantly different at p=0.006. The dorsomedial quadrant was significantly larger than its opposite quadrant p=0.0015.

Using the difference between complementary quadrants as an index of orientation, cells not near striosomes appear to be more strongly oriented than those close to them. Distant from striosomes, the components appear longer in the dorsomedial-ventrolateral axis. Closer to striosomes, there is a marked shorter component in the ventromedial direction.

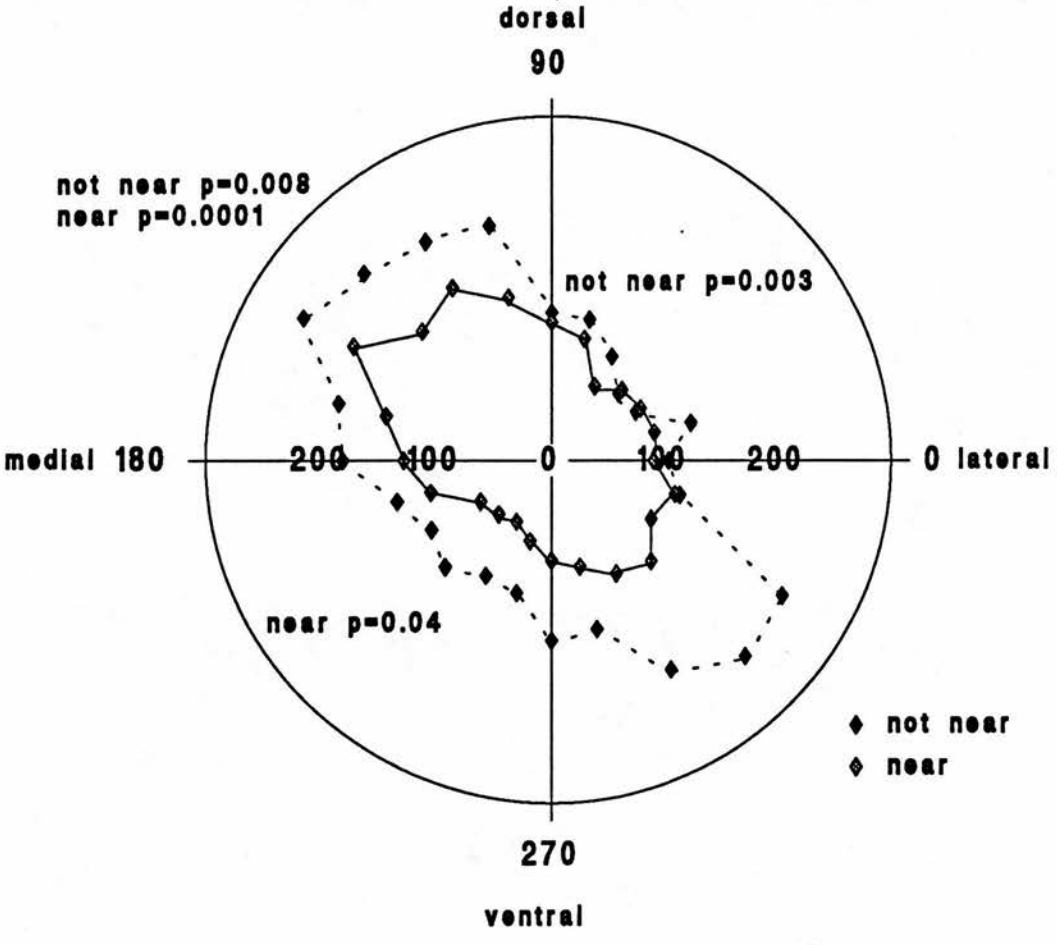

dorsal
90
not near p=0.008
near p=0.0001
not near p=0.003
medial 180
200
100
0
100
200
0 lateral
near p=0.04
not near
near
270
ventral

Figure 4.12 Cell bodies on deep sections (n=12)

Cells with their cell bodies on a deep section tended to have larger values for mean length of dendrite per segment as would be expected, p=0.008 (paired t test), but were similarly oriented to the group as a whole, with some small differences. From the figure it appears that for the cells on deeper sections, there are larger components along the long axis of the orientation.

Any differences between this group and the more superficial cells should be closely examined, to see if the data as a whole were biassed by loss of dendrites in a particular orientation.

The dorsomedial quadrant was significantly larger p=0.003; the ventromedial quadrant was smaller p=0.049.

The medio-dorsomedial octant was significantly larger, at p=0.0001 (p=0.0013 in all cells).

The ventro-ventromedial octant at p=0.04 (p=0.042 in all cells); complementary quadrants being significantly different at p=0.0004 (0.0001 in all cells).

There were no significant differences between the two pairs of diagonally opposing quadrants; in the group of all cells the two larger components, dorsomedial and ventrolateral differed p=0.016. This appeared to be due to an increase in the ventrolateral component in the deeper cells. Comparing the values for this quadrant in deeper cells versus those with more superficial cell bodies, using an ANOVA, the values for deeper cells were significantly larger, p=0.003.

The dorsolateral quadrant was not significantly smaller in this group, as it was (p=0.03) in all cells, and p=0.02 in superificial cells. The figure would suggest that this is due to a smaller sample size.

From the figure, the values for deeper cells are strikingly larger in both dorsomedial and ventrolateral directions. This suggests that there is no bias due to loss of dendrites in one direction more than the other.

Cells were filled on both sides of slices, however the cells on the rostral side of the slice were usually filled first, and this was often the better side, thus one would expect there to be an under-representation of caudal dendrites.

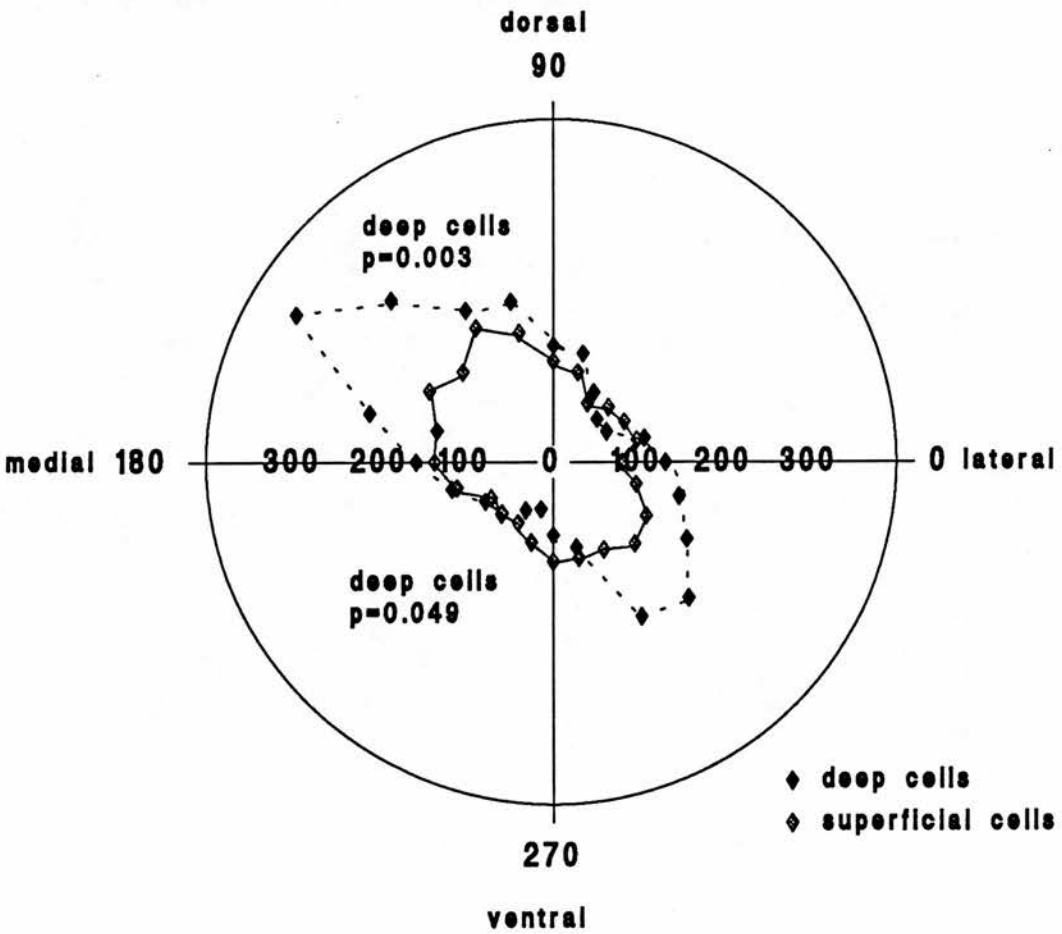

dorsal
90
deep cells
p=0.003
medial 180
300
200
100
0
100
200
300
0 lateral
deep cells
p=0.049
deep cells
superficial cells
270
ventral

Figure 4.13 Cells from mw6/17 (n=18)

The group of 18 cells from the same slice, mw6/17, showed a tendency to orientation identical to the rest of the coronal cells, although even more marked, especially in the dorsomedial quadrant p=0.0001 (p=0.0002 in all cells), and the medio-dorsomedial octant, p=0.0001 (p=0.0013 in all cells).

The dorsolateral quadrant was smaller p=0.04.

The complementary quadrants were significantly different p=0.0008 (p=0.0001 in all cells).

This suggests that any error due to malalignment between slices was small, and certainly did not affect significance.

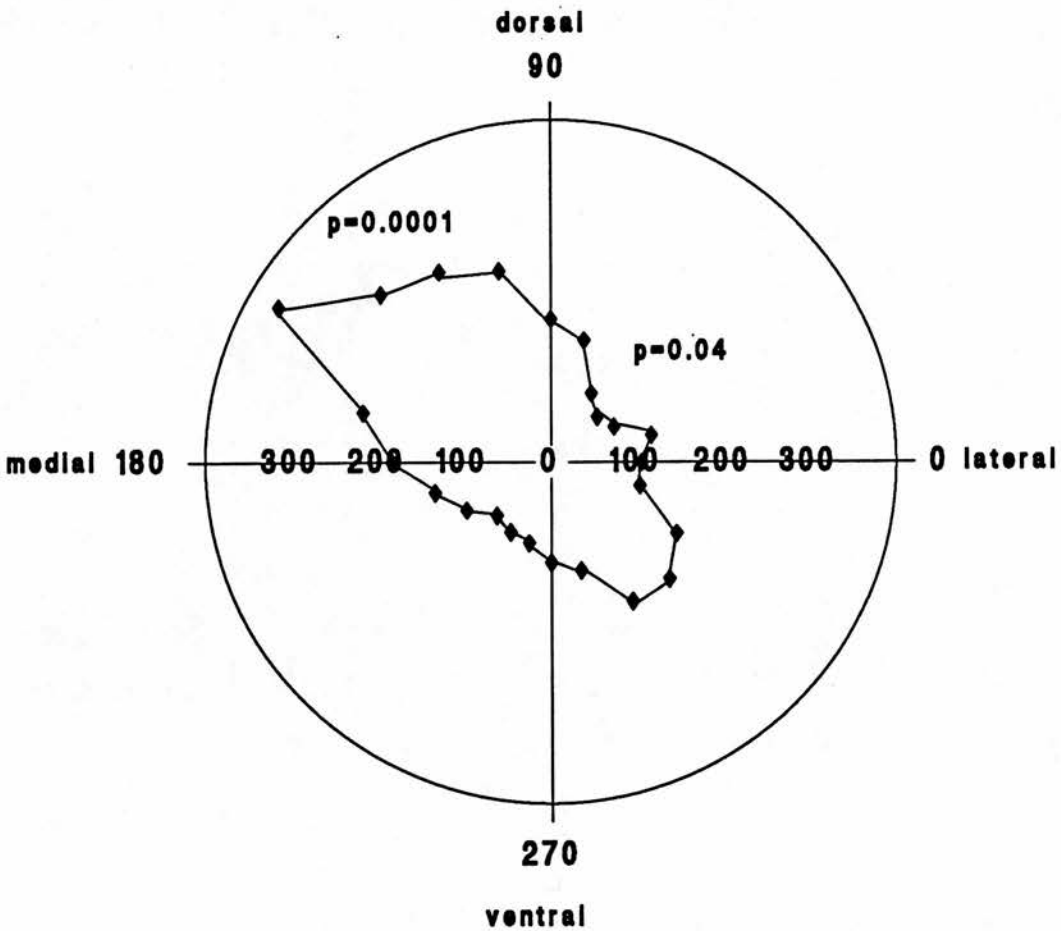

dorsal
90
p=0.0001
p=0.04
medial 180
300
200
100
0
100
200
300
0 lateral
270
ventral

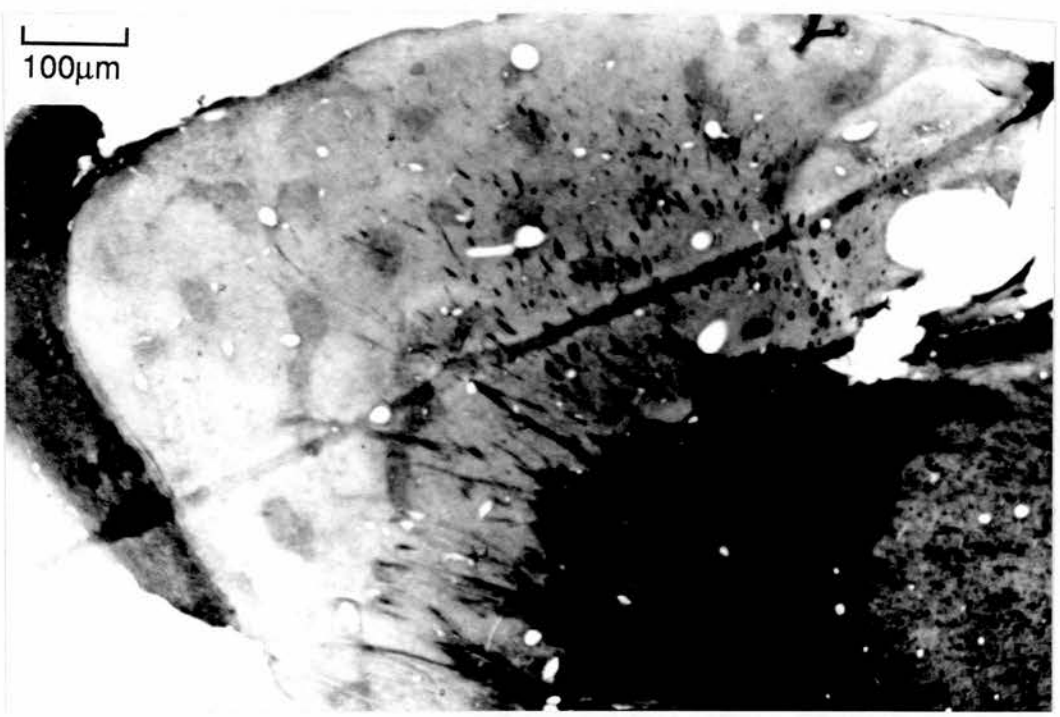
Figure 4.14 (a) Sagittal cells

The sample for this group was much smaller than for coronal cells.

The ventrorostral quadrant was significantly smaller p=0.02.

The ventro-rostroventral octant was significantly smaller p=0.044. The diagonally opposite quadrant was not different from the rest, and the value for complementary quadrants was p=0.058.

(b) Sagittal section oriented as diagram opposite



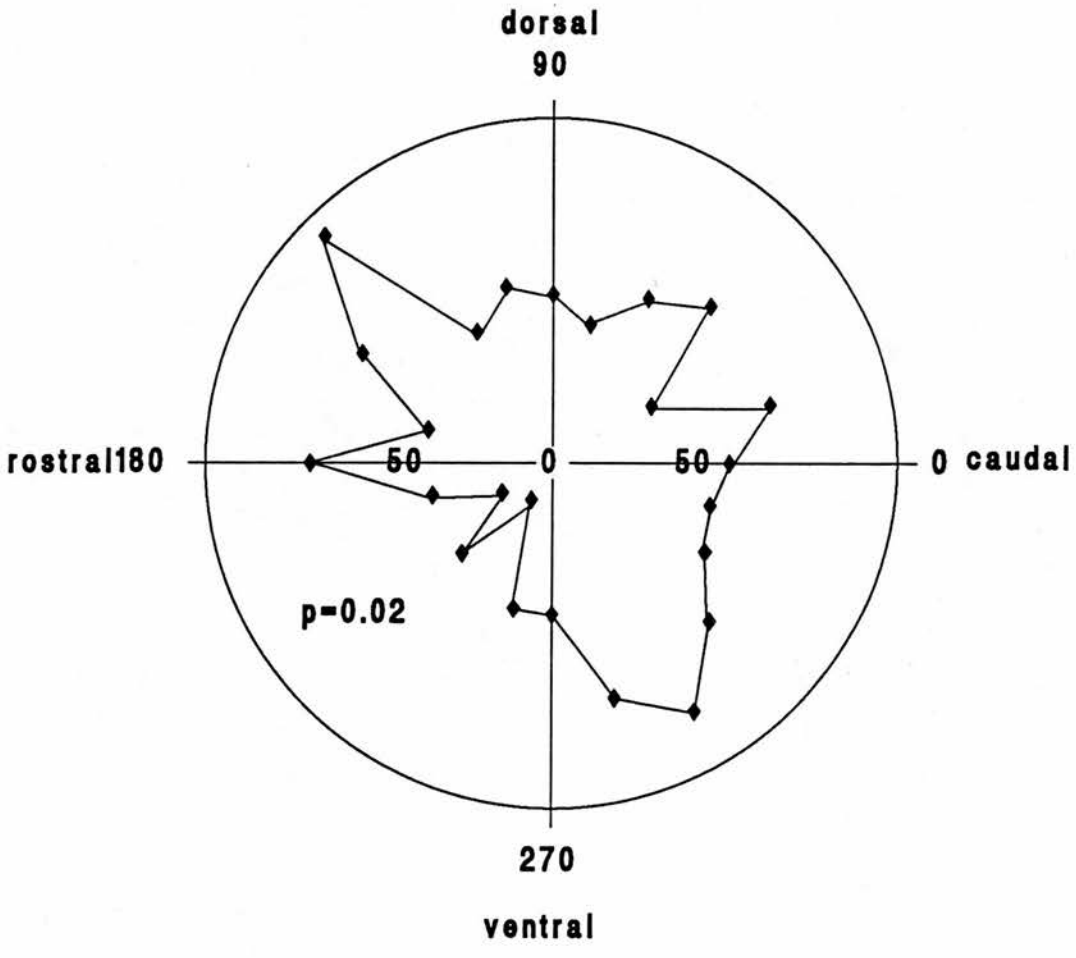

dorsal
90
rostral180
50
0
50
0 caudal
p=0.02
270
ventral

Figure 4.15 Orientation of dendritic fields in three dimensions; coronal and sagittal views from squirrel monkey, horizontal view from ferret

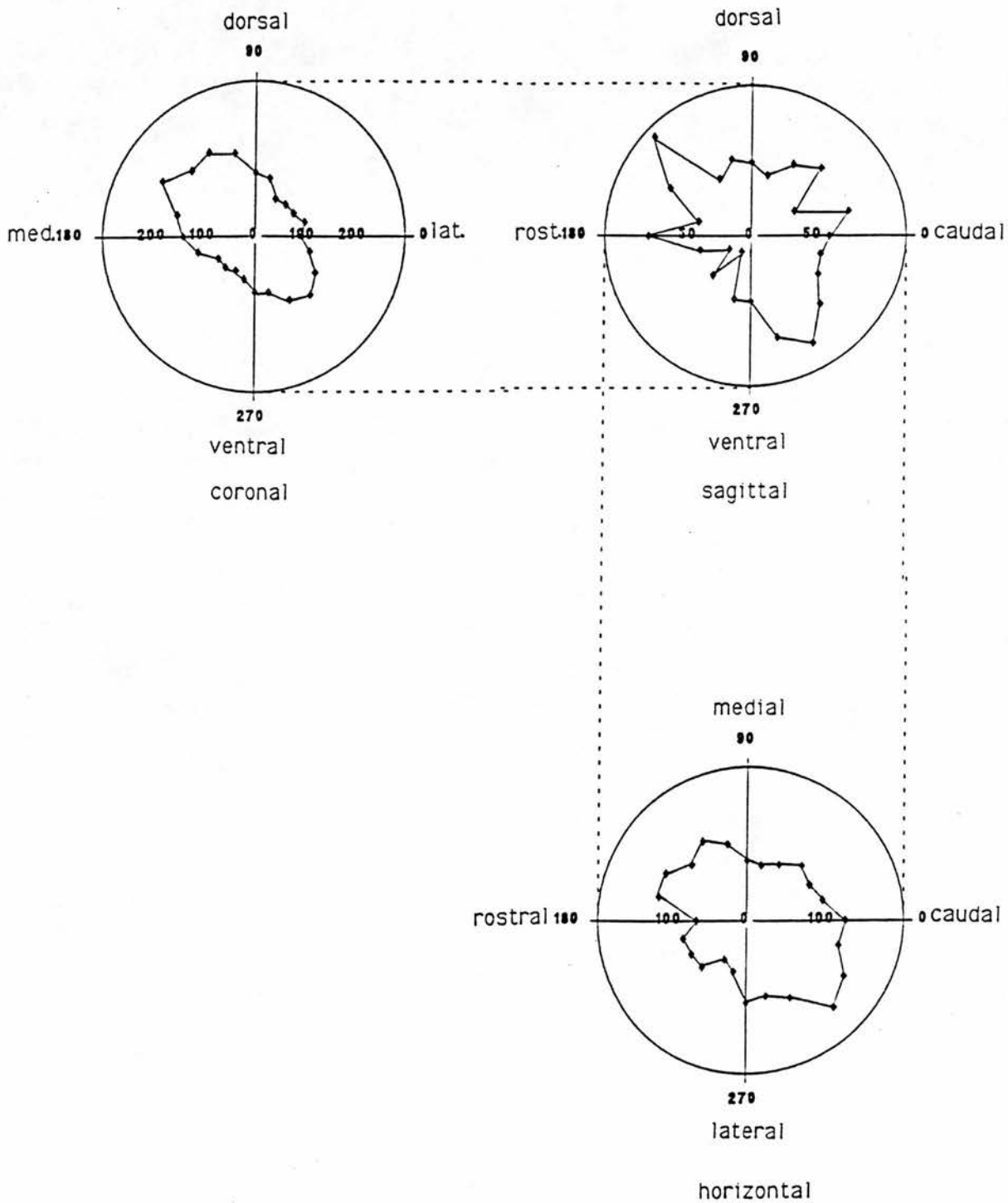
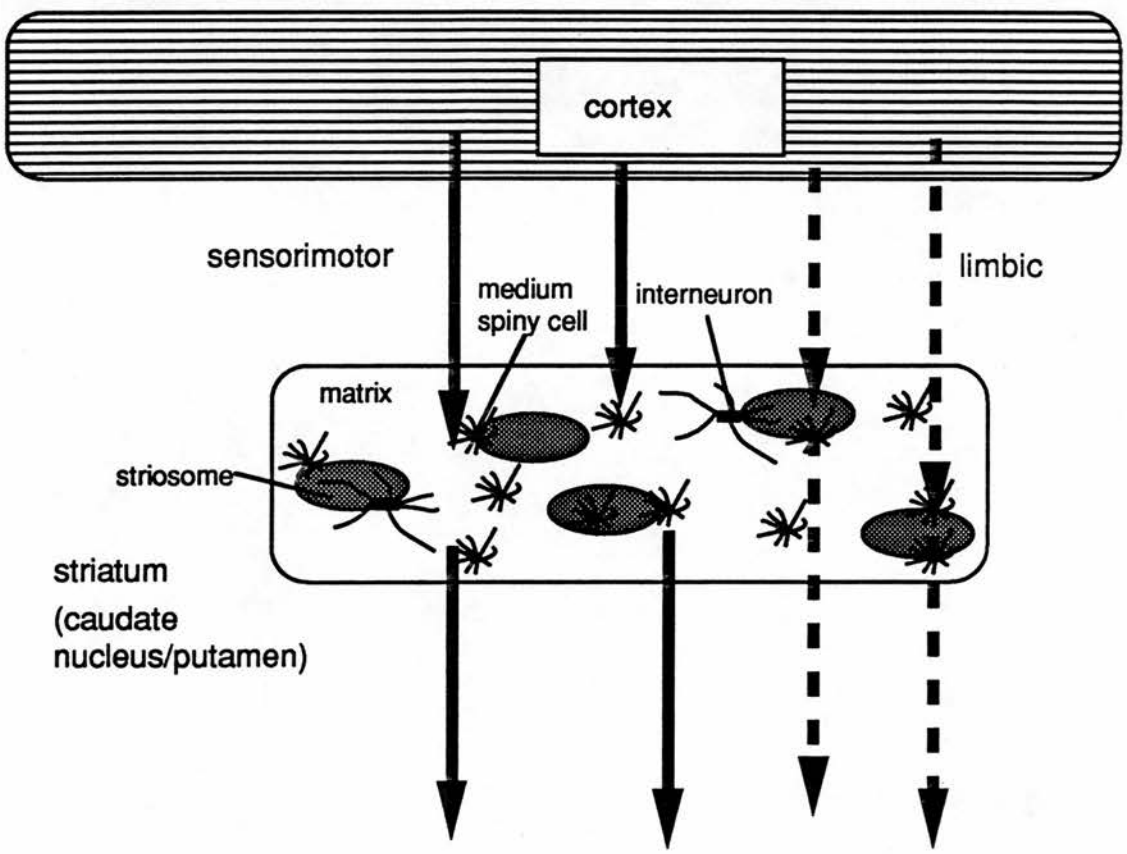


Figure 4.16 Striatal efferents can be directly influenced by inputs to both compartments



Chapter 5

Concluding discussion

Analysis of the data presented in Chapters 3 and 4 leads to two conclusions about the anatomical organisation of the caudate nucleus which have implications for the understanding of basal ganglia function.

Recent thinking about the organisation of the basal ganglia has focussed upon the idea of a number of parallel loops (see Alexander and Crutcher, 1990). Within the striatum these pathways would pass through either the striosomes or the matrix, depending upon their extrastriatal connections. The majority of afferent pathways make direct synaptic contact with the medium spiny cells, which are the source of efferents from the striatum. Information to date has implied that the two compartments of the striatum and their respective extrastriatal connections are segregated, with communication between the two being carried out by interneurons.

The work presented here suggests that there can be considerable overlap of the striosomal borders by the medium spiny cells. This would mean that afferents to one compartment could have direct influence over the other to the extent that local dendrites transgressed the border. If the interneurons were the only cells communicating with both compartments, the neuronal information passing through one compartment would reach the other only after being modified by the interneuron.

Although the percentage of medium spiny neurons with dendrites crossing striosome/matrix borders is only about 10%, this is a larger proportion of the neuronal population than the interneurons, which make up 4% of the striatal cell population. Of these, only the large cholinergic neurons have dendrites which are much longer than those of the medium spiny cells. This would suggest that the medium spiny cells crossing borders play a significant role in integration of striatal function.

The papers by Kawaguchi *et al.* (1989) and Penny *et al.* (1988) demonstrated local axon collaterals remaining primarily within the compartment in which their cell body was. The results presented here would mean that the process of lateral inhibition, or any other type of local interaction, between medium spiny cells by means of local axon collaterals would not be restricted, but would affect cells of the other compartment to the extent that their dendrites crossed the border.

In interpreting this data in relationship to striosomes, it is important to do this in the context of how they are defined. As discussed in Chapter 2, there are many techniques which produce this pattern of labelling. Some markers such as antibodies to substance P clearly stain cell bodies and dendrites which define striosomes. Others, such as antibodies to enkephalin, will label

either striosomes or matrix depending upon the protocol used. *In situ* hybridisation studies, reflecting the capability of the neuron to produce these peptides, show a large overlap in subpopulations. Tracing studies provide strong evidence that this compartmentalisation has functional significance; the segregation of pathways from different sites, together with receptor binding studies, implies that there is a separation of neuronal information at this level.

The data presented here also demonstrate that the dendritic arborisations of medium spiny projection neurons are strongly oriented in the ferret and squirrel monkey caudate nucleus. The long axes of the dendritic trees appear to be parallel to those of most striosomes. This is not surprising in that the neuropil of these cells comprises many elements of the histochemically defined striosomes, but it has not been previously described. However, it is not just in the vicinity of the striosomes that the cells are strongly oriented; this feature is seen just as clearly in the cells located in the matrix distant from striosomes.

Graybiel and co-workers (1991) demonstrated, using anterograde labelling from motor cortex, that this projection splits up to label discrete areas of matrix, similar to but distinct from striosomes, which they call "matrisomes". They also observed clustering of projection neurons (Gimenez-Amaya and Graybiel, 1991),

which appear to show the same organisation. Crutcher and Delong (1984) recorded from cells within the primate putamen which responded to peripheral stimulation, and found that they tended to be topographically organised into clusters of cells, recorded over 100-500um, responding to the same stimulus. Also working in the primate putamen, Alexander and DeLong (1985a,b) describe discrete "striatal microexcitable zones", stimulation of which evoked movements. These zones ranged from 200-1200um in width, usually within the range 300-800um. It might be that the orientation of dendritic arborisations seen here is related to these areas; the length of dendrites of medium spiny neurons of most species ranges from 200um to 300um (Dimova *et al.*1980; Graveland *et al.*1985a).

Kawaguchi and colleagues (1990) noted "recurved endings" of distal dendrites in order to avoid crossing a striosome border. I did not observe these in the dendrites of cells distant from striosomes. This suggests that whatever structures lie within the matrix, their borders are not so clearly defined in terms of dendrites of medium spiny neurons as those of striosomes.

The dorsomedial-ventrolateral orientation is also seen in the shape of terminal fields of various corticostriatal projections. This was demonstrated in

experiments mapping the representation of cortical areas corresponding to body parts in the caudate-putamen (Kunzle, 1975; Percheron *et al.*1984) (figure 5.1(a)). There is evidence for a longitudinal component to the corticostriatal projection (figure 5.1(b),(c)). This has been observed in rats by Jones and co-workers (1977) and by McGeorge and Faull (1987, 1989). This was also seen in projections from auditory cortex to striatum in the cat (Reale and Imig, 1983). Brown (1991) mapped corticostriatal connections in rats, observing 2-deoxyglucose activation in the striatum following peripheral sensory stimulation. She found that the areas activated for each peripheral site formed strips oriented from rostromediodorsal to caudolateroventral. There were variations in the relative positions of areas from different body parts, permitting interactions between all components. The input from cortex would correlate with the orientation of striatal medium spiny cells, permitting a match of input and output with relatively little overlap with adjacent areas. This organisation is disrupted in the hemiparkinsonian rat (L.L. Brown, personal communication) which suggests that dopamine is essential in localising the activation produced by a cortical input.

The length of the dendrites of medium spiny cells, 200-300um, is such that integration of information from a large area of the striatum is not likely to be a major

function (c.f. Percheron *et al.*1984). However, the number of spines upon the dendrites greatly increases the area available to make synapses (Wilson, 1991), and it has been suggested that inputs from a number of different cortical cells make contact with dendritic spines over a short length of dendrite (Somogyi *et al.*1981; Groves, 1983). The contribution of a single input will be minor in this case, and the striatal cell will be primarily sensitive to coordinated inputs (Wilson, 1991). In most cases these would come from topographically-related cortical cells, but if, for example, a cell had dendrites extending out of its matrisome into a nearby striosome, it could be significantly influenced by inputs from other sources.

In a study of striatal afferents and efferents using small localised injections of tracers into the primate striatum, Hedreen and Delong (1991) comment that it was difficult to label cortical neurons, and they surmise that this was due to the fact that the terminal field of each cell is distributed over a large area. Certainly, extensive collateralisation of corticofugal axons have been seen in the rat (Donoghue and Kitai, 1981). This would lead to one cortical neuron making synaptic contact with many medium spiny cells, probably all within one topographically-specified domain.

The compartmentalisation of the striatum into striosomes

and matrix corresponds to some extent with a division of "limbic" and "sensorimotor" functions. However, this seems to be increasingly less significant in correlating extrastriatal connections with the intrastriatal histochemical distinction. Ragsdale and Graybiel (1990), studying projections from different regions of cortex, noted that whilst borders were still observed, in ventral regions the correspondence was often reversed. The dorsoventral level at which the reversal occurs varied with the region of cortex, and was always more ventral in lateral regions. This implies yet another dimension to the organisation of corticostriatal connections which follows the same orientation.

An alternative conclusion is that, whilst striosomes are a factor in striatal organisation, there is some other more significant element, which generates the strong directional component of all medium spiny cells, whether they are in the vicinity of a striosome/matrix border or not. The terminal fields of afferents and the cell bodies of efferents seem to be organised in effectively parallel domains throughout the striatum. Interactions between cells relating to, for example, different parts of the body, would be determined by the physical relationships between the strips, which vary with position in the striatum.

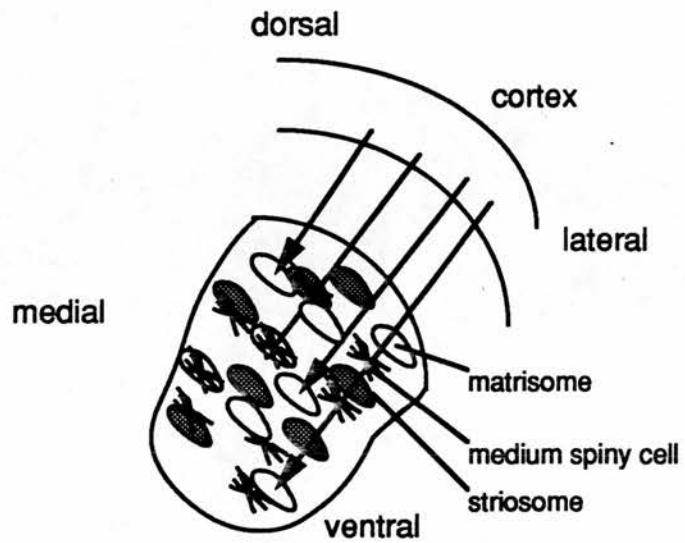
The anatomical organisation seen here might be due to developmental factors, whether there is a functional

correlation or not. Radial glial fibres appear to be important in prenatally determining the location of neurons (Rakic, 1972). Some authors have postulated that this is not such an important factor in the striatum as it is in the cortex (Johnston *et al.*1989, 1991). However, it has been shown in embryonic tissue from the mouse (Edwards, 1991) and the rat (Liu and Graybiel, in press) that there are radial glial fibres which are aligned with the corpus callosum. Neurons migrate along these to their final positions. If the alignment of radial glial fibres in the species examined here was appropriate, this might explain the orientation of matrix cells. If the cell body remains oriented with its long axis along the radial glial fibre, its dendrites would grow out with the same orientation. In tissue from the above studies there is no evidence of this behaviour, but it is possible that these patterns could appear at a later date.

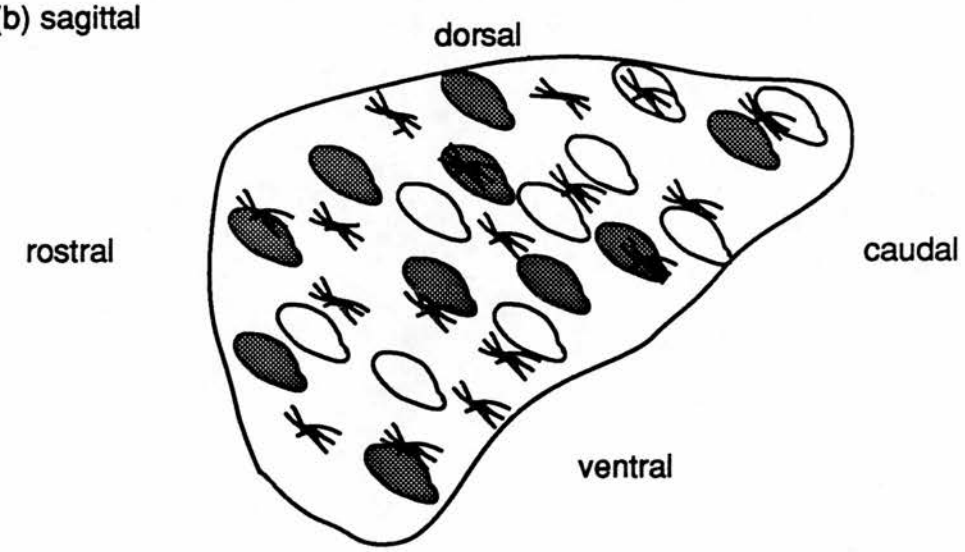
In conclusion, the method developed here has permitted an examination of the dendritic arborisations of the medium size spiny projection neurons of the primate caudate nucleus. They are not strictly confined by striosome/matrix boundaries, although there is a clear interaction with these elements, and they appear to be influenced in their orientation by additional factors as yet unknown. These results suggest a new level of functional organisation of the striatum.

functional organisation of the striatum.

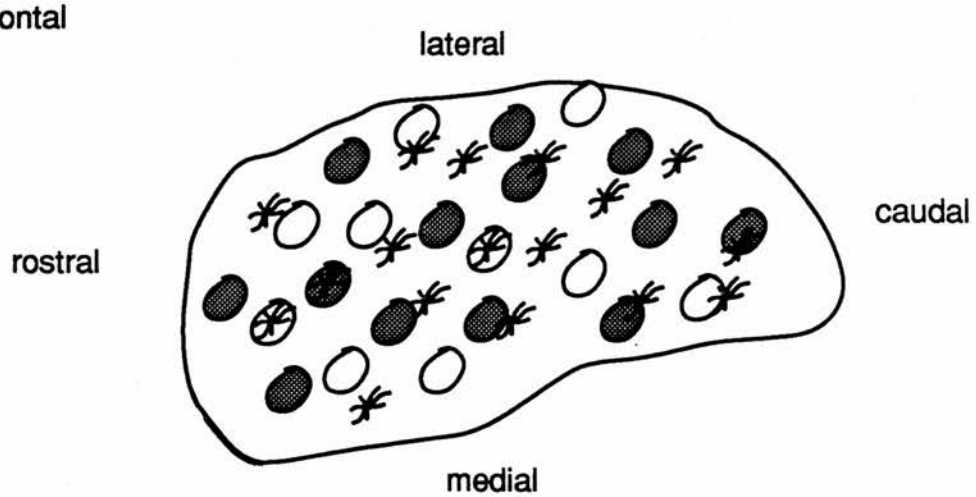
Figure 5.1 Proposed organisation of the striatum
(a) coronal



(b) sagittal



(c) horizontal



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Further evidence for a pallido-striatal pathway in rat brain

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The first direct evidence for the existence of pallido-striatal neurones came from experiments in which cells in globus pallidus (GP) accumulated horseradish peroxidase-wheatgerm agglutinin conjugate after its injection into the neostriatum (Staines, Atmadja & Fibiger, 1981). Since this method did not give information about the neurochemical and neurophysiological properties of the pathway we have studied it in two other ways.

Male albino Wistar rats (approx. 200 g) were used in all the experiments. Thirty-seven animals were anaesthetized with Equithesin and 0·2 μl. of fluorescent dyes (True Blue, Nuclear Yellow) stereotaxically injected into the striatum through a 30 gauge needle. 24–72 hr later the animals were re-anaesthetized and perfused transcardially with phosphate buffer followed by 4 % paraformaldehyde dissolved in the same buffer. The brains were removed, post-fixed in the same solution overnight and then left in 20 % sucrose for at least 24 hr.

In frozen sections cut from brain prepared in this way it was possible to detect cells in the GP which had accumulated dye, even in animals in which the dye was co-injected with kainic acid (2 n-mole). Since kainic acid is known to block orthograde transport this suggests that stained cells had transported dye retrogradely from terminals in the striatum. Sections from ten of the animals were also prepared for immunohistochemistry with antibodies to either Leu- or Met-enkephalin or to endorphine. No retrogradely labelled cells were stained with any of the antisera.

In a separate series of fifty animals extracellular recordings were made from the region of GP in rats anaesthetized with halothane. A concentric stimulating electrode was placed in the neostriatum and the responses of the units detected were recorded on magnetic tape by a microcomputer system designed and built in the Unit around an F100-L processor. Of the 102 cells which were recorded from histologically identified sites in the GP, eighty-one were driven from the stimulus and fifty-four were spontaneously active. Of the thirty-three units in both of these classes the evoked activity was unlikely to be antidromic in twenty-six. The seven cells in which spontaneous action potentials could be shown to collide with the driven response had latencies of activation ranging from 2–5 msec, giving conduction velocities in the region of 0·3 m/sec.

These experiments provide confirmation of the existence of the pallido-striatal cells described in previous anatomical experiments.

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Pallido-striatal neurones with branches to the mesencephalon – electrophysiological evidence in the rat

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In a recent communication to the society (Arbuthnott, Walker, Whale & Wright, 1983) experiments were described which provided electrophysiological and neuroanatomical evidence for pallido-striatal neurones in rat brain. Further anatomical investigations suggest that the same neurones also project to the substantia nigra (SN). The lectin prepared from phaseolus vulgaris (PhA.L – Vector Laboratories) is taken up and transported anterogradely by neurones (Gerfen & Sawchenko, 1982). Following ionophoresis of PhA.L into globus pallidus (GP) terminal fields in SN as well as in neostriatum are seen (Staines, personal communication).

We attempted to provide evidence for the existence of GP neurones with axons projecting to neostriatum and also down the crus cerebri through which pallido-nigral fibres are known to project. Ten male albino Wistar rats weighing 200–250 g were anaesthetized with halothane and a systematic search of the GP region was made with glass micro-electrodes. Stimuli were applied to the striatum and the crus cerebri through concentric stimulating electrodes.

Extracellular units were recorded in GP that responded to stimulation by either or both electrodes. Antidromic activation was identified by the collision test. Twenty-two units were activated from the electrodes and shown at subsequent histology to be within GP. Five of them responded antidromically only to stimulation of the neostriatum, eight to stimulation of the crus cerebri; five were antidromically driven by both electrodes and only three cells did not show antidromic activation in this sample.

Thus some units recorded in GP project both to striatum and towards SN. Because the pallido-striatal pathway is topographically organized and because the stimulation may not have activated all the descending fibres in the crus cerebri a larger proportion of GP cells may project in both directions than have been detected. All the units which were antidromically activated from the crus cerebri alone were orthodromically activated by striatal stimulation with variable latency (2–25 ms).

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Experimental Brain Research

Electrophysiological and anatomical observations concerning the pallidostriatal pathway in the rat

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Summary. Anatomical studies in several species have demonstrated a pallidostriatal pathway. We employed electrophysiological and anatomical methods to distinguish the neurones of this pathway from the two pallidocortical groups reported in the rat. Injection of fluorescent retrograde tracers, combined with immunohistochemistry for choline acetyltransferase, provided anatomical evidence of the distinction between neurones of the cholinergic pallidocortical projection and pallidal cells retrogradely labelled from the striatum. Extracellular recordings made in the globus pallidus of halothane-anaesthetised rats provided an electrophysiological description of the pallidostriatal pathway. In some animals neurones of this pathway were distinguished from pallidocortical neurones as a stimulating electrode, situated in the crus cerebri, permitted identification of neurones which projected through this region as well as to the striatum. Neither pallidocortical pathway is reported to have descending axons travelling in the crus cerebri at this point. A preliminary electrophysiological study was carried out in rats in which the dopamine-containing cells of substantia nigra had been destroyed by injections of 6-hydroxydopamine at least 6 months prior to the recording. In the globus pallidus on the lesioned side the mean firing rate of neurones was increased compared with controls. No specific change in firing pattern was noted but the neurones were more responsive to striatal stimulation suggesting that long-term dopamine denervation alters the sensitivity of neurones of globus pallidus.

Key words: Globus pallidus – Pallidostriatal neurones – Cholinergic neurones – Dopamine – 6-hydroxydopamine

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Introduction

The globus pallidus (GP) plays an important part in the hierarchy of the basal ganglia involved in the processing of motor commands. It receives input from the neostriatum and the subthalamic nucleus and is known to project to the ventral thalamus, subthalamic nucleus, substantia nigra and ventral tegmental area (Nauta and Mehler 1966; Graybiel and Ragsdale 1979). The influence of the striatum upon GP is for the most part inhibitory (Levine et al. 1974). Each pallidal cell has a dendritic field large enough to receive input from a very large number of neostriatal cells and thus the GP has been proposed to be involved in a filtering or focussing type of action (Percheron et al. 1984).

The possibility of a reciprocal pallidostriatal connection was noted first by Wilson in 1911, and later by Mettler in 1943. Autoradiographic studies by Nauta (1979) provided more concrete evidence for this projection, and Staines and co-workers (1981) demonstrated pallidostriatal connections using retrograde transport of horseradish peroxidase (HRP) conjugated to wheat germ agglutinin. Recently Jayaraman (1983) used retrograde transport of HRP to illustrate a topographical relationship between GP and striatum. Such an ordering of the pallidostriatal and pallidonigral pathways has been related to the known striatonigral topography by Staines and Fibiger (1984). GP cells with collaterals to substantia nigra and striatum could thus modulate neuronal activity in these two nuclei.

There is evidence that in the rat some rostrally-projecting pallidal cells have collaterals to the paraventricular nucleus of the thalamus which, in its turn, projects to the nucleus accumbens (Takada et al. 1986); these cells may play a role in the integration of motor and limbic systems. Small cells in GP may also project to cerebral cortex (van der Kooy and Kolb

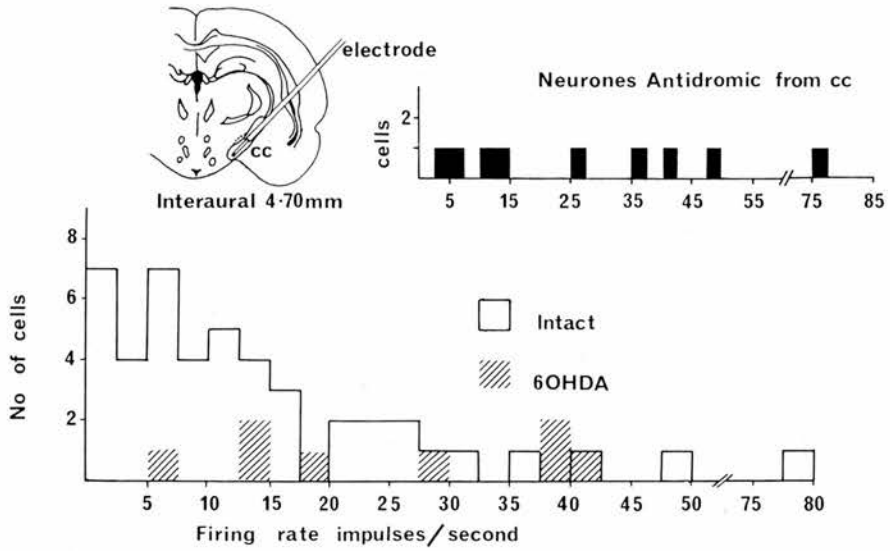

Fig. 1. The distribution of firing rates seen among the spontaneously active neurones encountered in the globus pallidus of anaesthetised rats. The hatched bars represent the firing rates of neurones isolated in GP from a separate group of animals treated 6 months previously with 6-OHDA, Although the rates seen are within the normal range they are faster on average ($p < 0.01$ Mann-Whitney 'U' test). Above is plotted the stimulation site in the crus cerebri and a histogram separately illustrating those cells which were spontaneously active and antidromically driven from the electrode position shown. These cells also form a distinguishably faster group. Interaural 4.7 – plane of the Paxinos and Watson (1986) atlas from which the diagram was drawn. cc – crus cerebri

1985) but these, unlike the pallidostriatal cells, do not have descending axons.

We aimed to carry out an electrophysiological investigation of the neurones of the pallidostriatal pathway which, in separate experiments, we had identified by retrograde transport of a fluorescent dye. As van der Kooy and Kolb (1985) had demonstrated pallidocortical cells which did not project caudally, we could confirm the identification of pallidostriatal cells by stimulation of their axons in the crus cerebri. Some preliminary experiments were carried out in rats which had received 6-hydroxydopamine (6-OHDA) injections into the medial forebrain bundle, in order to study the effect of the long-term loss of dopaminergic control of striatal cells upon pallidal neurones.

Methods

Neurophysiology

Sixty-four albino Wistar rats (200–250 g) were anaesthetised with halothane, tracheostomised and placed in a stereotaxic frame on an electrically heated blanket with a rectal probe to control body temperature.

A concentric stimulating electrode was positioned in the anterior striatum (1 mm anterior and 2.5 mm lateral to the Bregma suture and 5 mm vertically below the surface of the dura). In 10 animals a second stimulating electrode was placed in the crus cerebri (4 mm posterior, 2.5 mm lateral to Bregma and 7.8 mm vertically below dura membrane). Glass pipettes were filled with 2% Pontamine Sky blue in 0.5 M sodium acetate which gave resistances of 20–60 MΩ (measured at 1.5 Hz). Extracellular recordings were made from electrode tracks aimed at the GP (0.8 mm posterior and 2.5 mm lateral to the Bregma suture). Individual neurones were isolated either because of their response to electrical stimulation by one of the implanted electrodes, or because of their resting activity. Interspike interval and post-stimulus histograms were generated on-line with a computer (Arbuthnott et al. 1984), and data was stored for further analyses. Antidromic responses to either electrode were identified by collision of evoked activity with spontaneous action potentials. The positions from which recordings were made were marked by passing a negative current (5 μA for 5 min) through the electrode to deposit the Pontamine Sky blue. The experiment was terminated after a maximum of three tracks; the animal was killed with a lethal dose of anaesthetic and the brain removed and frozen. Sections 40 μm thick were cut on a cryostat, mounted, and stained with various 'Nissl' stains in order to determine the position of the stimulating electrodes and the marked recording sites. This histological verification determined which results were included in the final analysis of data.

Records were also obtained from 7 animals which had received 6-OHDA injections (8 μg in 4 μl) into the medial forebrain bundle at least 6 months previously. When tested 5–9 days after the 6-OHDA injection each animal turned more than 200 times contralaterally to the lesion during 45 min following administration of apomorphine (0.25 mg/kg, i.p.). These animals can be expected to have a depletion of more than 90% of dopamine from the striatum on the lesioned side (Hefti et al. 1980).

Neuroanatomy

12 male albino Wistar rats (200–250 g) were used for the anatomical part of the investigation. Under Equithesin anaesthesia (16% Nembutal 4.2% chloral hydrate in a polyethyleneglycol : alcohol : water mix; 3 ml/kg, i.p.) stereotaxic injections of 2% True Blue were made into the striatum in 8 rats. A total volume of 0.2 μl was delivered through a 1 μl Hamilton microsyringe, at co-ordinates 1 mm anterior and 2.5 mm lateral to the Bregma suture and 5 mm vertically below the dura membrane. In order to reduce spread up the needle track the injections were made over 5 min and the syringe was left in place for another 2 or 3 min before being slowly removed. 4 rats received iontophoretic injection of 2% fluorogold (Fluorochrome Inc; Colorado) into the striatum at the same co-ordinates from a 10–20 μm-tipped glass micropipette.

After survival times of 4–16 days, the rats were given a lethal dose of anaesthetic and perfused through the heart with 0.1 M sodium phosphate buffer (pH 7.4) followed by 4% paraformal-

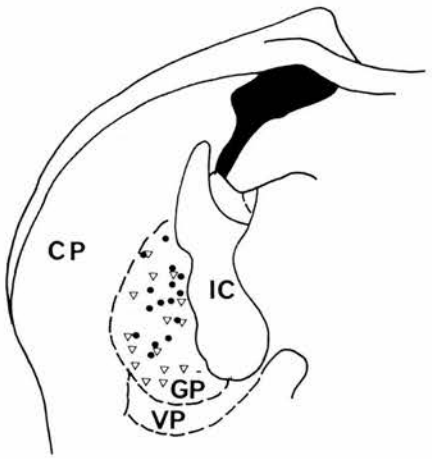

Fig. 2. A drawing from part of the coronal section through the interaural 7.7 level in the Paxinos and Watson atlas, showing the distribution of the 15 fastest and 15 slowest neurones recorded. Neurones marked (▽) all fired slower than 5 impulses/s, while those marked (●) fired above 24 impulses/s. IC – internal capsule, CP – striatum (caudate/putamen), GP – globus pallidus, VP – ventral pallidum

dehyde in the same buffer. The brains were removed, stored in the same fixative overnight, and then transferred to 20% sucrose for at least 24 h. Sections 50 μm thick were cut on a freezing microtome and collected in 0.5 M Tris buffer (pH 7.4). For some animals, alternate sections were mounted on chrome alum/gelatin coated slides, air dried, and examined under a fluorescence microscope. The parallel set of sections was stained for choline acetyltransferase (ChAT) by incubating overnight in a monoclonal antibody (a gift from Dr. F. Eckenstein) 1 : 4 in 100 mM Tris buffer (pH 7.4) including 150 mM sodium chloride, 2% bovine serum albumin, 10% rabbit serum, 0.5% Triton X-100, and 0.1% sodium azide. They were then rinsed in the same buffer and incubated for 30 min in biotinylated rabbit anti-rat IgG (Vector). The rabbit secondary antibody was then visualised by reacting the sections for 30 min with a mixture of Avidin DH combined with biotinylated R-phycoerythrin. The last two incubations were repeated, and then the sections were mounted on chrome alum/gelatin coated slides, dried in air and studied with a fluorescence microscope (Zeiss) with appropriate filter sets for fluorogold and the phycoerythrin.

Results

The distribution of firing rates among the 55 spontaneously active neurones recorded from 27 rats is shown in Fig. 1. Nine neurones recorded from the 6-OHDA lesioned animals are also included. The firing rates of spontaneously active GP neurones ranged from 0.6 to 79 impulses/s (median 13.9). There did not seem to be topographical distribution of cells according to firing rate; Fig. 2 illustrates the positions of the 15 slowest and the 15 fastest neurones encountered. Nevertheless, groups of neurones recorded sequentially in any one track often had strikingly similar properties. Figure 3 selects a track in which the firing pattern of each neurone encountered in response to striatal stimulation is illustrated alongside its position within GP.

Thirty-two of the 55 spontaneously active neurones were antidromically activated from the striatum. The mean latency was 2.9 ± 2.1(SD) ms

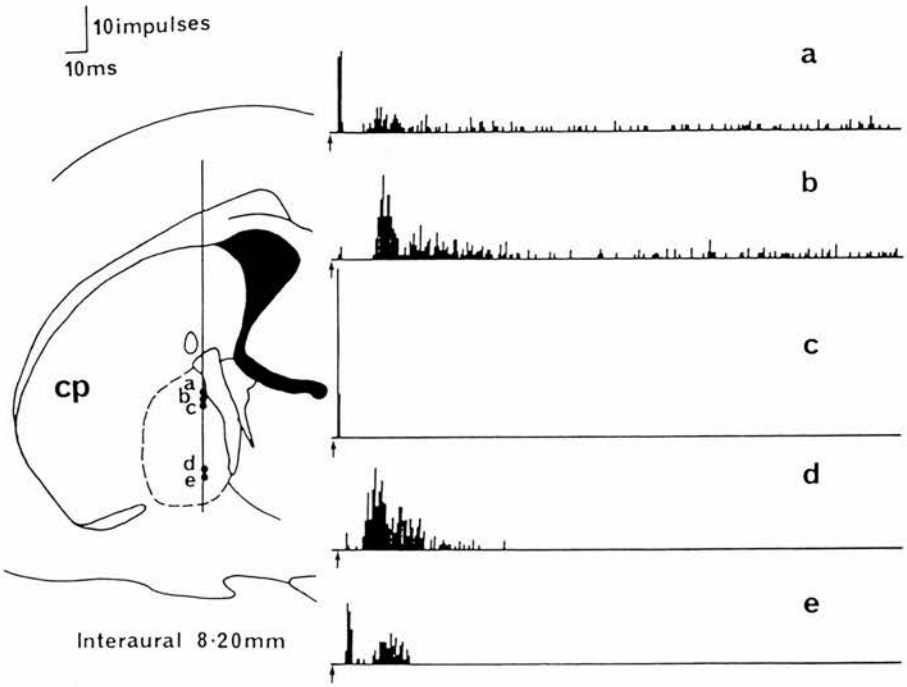

Fig. 3a–e. Poststimulus histograms on the right of the figure illustrate the responses to 500 stimuli delivered to the striatum at 1 Hz. They were recorded at positions in the brain indicated by the equivalent letters on the reconstruction of the electrode track through the GP, drawn on the left. Neurones with broadly similar responses tend to cluster together (e.g. **a, b; d, e**). The anatomical drawing is based on the rat brain atlas (see Fig. 2 for details)

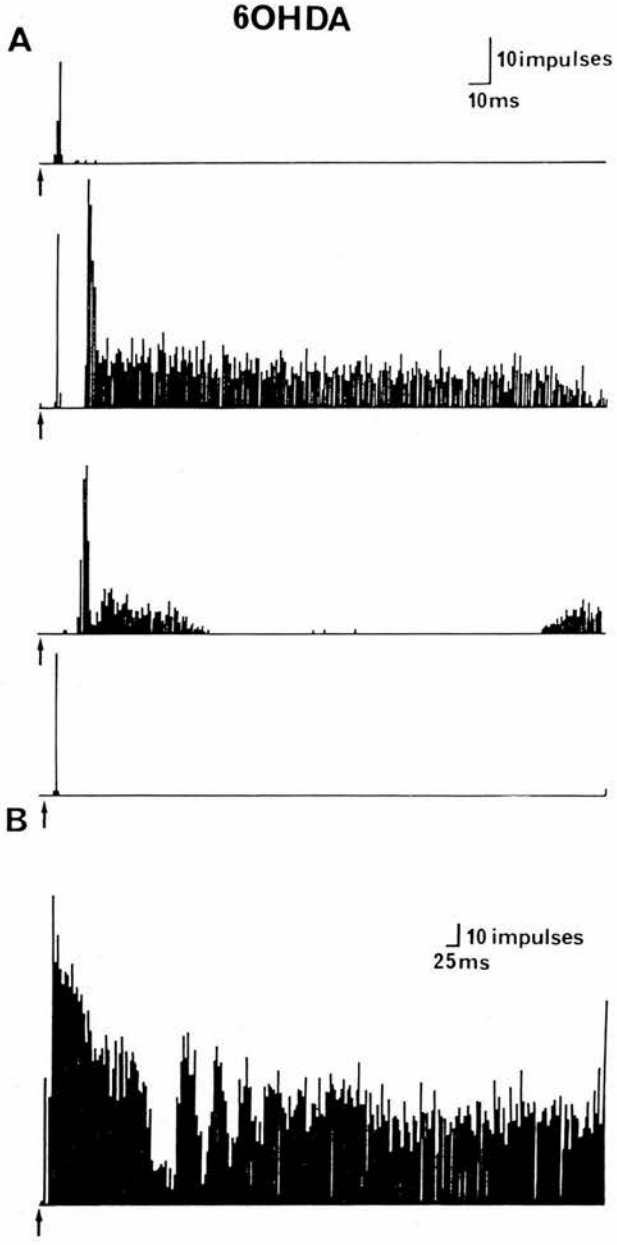

Fig. 4A, B. These post stimulus histograms are similar to those in Fig. 3 except that they come from two rats previously treated with 6-OHDA. Many neurones are hyperresponsive to the striatal stimulus applied at the arrow. Note the change of scale for part B

with a collision interval of 3.2 ± 1.9 ms. Twelve of these neurones were recorded from the 10 animals with stimulating electrodes placed both in the striatum and in the crus cerebri. Five neurones were antidromically driven from both areas and antidromic activity evoked only by crus cerebri stimulation was observed in the remaining 7 neurones. The firing rates of the 12 neurones which were thus identified as having axons in crus cerebri ranged from 0 to 76.6 impulses/s (median 25.9, mean ± SD 22.1 ± 16.5), which is significantly higher than for the other GP neurones ($p < 0.01$, Mann Whitney U test).

All the antidromic activity reported was confirmed by collision with spontaneous action potentials. None of the silent neurones excited from the striatum ($N = 38$) were therefore included although they often followed high frequencies of stimulation and occasionally had short, reasonably constant latencies (< 2 ms variability). We did not see any sign of orthodromic activity with a timescale of milliseconds driven by the crus electrode although long term changes (over several seconds) in firing rate were sometimes seen.

In the preliminary experiments on 6-OHDA treated animals lesions were made at least 6 months earlier. Nine neurones, from 3 lesioned rats, were recorded in the GP ipsilateral to the lesioned side. These neurones fired significantly faster ($p < 0.01$ Mann-Whitney U test), although they all lie within the wide range found in normal animals (Fig. 1). Another important characteristic of these neurones, illustrated in Fig. 4, is that they were markedly more responsive to striatal stimulation than were cells recorded in unlesioned rats. A particularly striking example is shown in Fig. 4B; this kind of multiple response was seen only in lesioned rats and never in control animals.

In each animal with a successfully placed striatal injection of either True Blue or Fluorogold, retrogradely-labelled cell bodies were found in the GP. In the anterior portion of the nucleus the neurones were located laterally, just within the pallidostriatal border. In middle portion of the GP, cells were arranged both laterally and more medially within the nucleus. At more caudal levels, within GP behind the anterior commissure (1 mm caudal to Bregma), the medial group of cells was still visible, while neurones labelled in the lateral zone were scarce. There were usually no cells in the entopeduncular nucleus, although we often found some cell bodies among the fibres of the internal capsule immediately caudal to GP. Figure 5 plots the results from typical successful True Blue injection.

When small injections of tracer were localised to the striatum no retrogradely-labelled pallidal cells were double-labelled with ChAT. If the injection of the retrograde label was bigger, and especially if it leaked to cortex, a wider distribution of labelled cell bodies was seen. Figure 6 illustrates common features of a section from a case in which the injection site included some leakage to cortex. Neurones are visible below the ventral limits of GP within "ventral pallidum"; some of these are double-labelled with

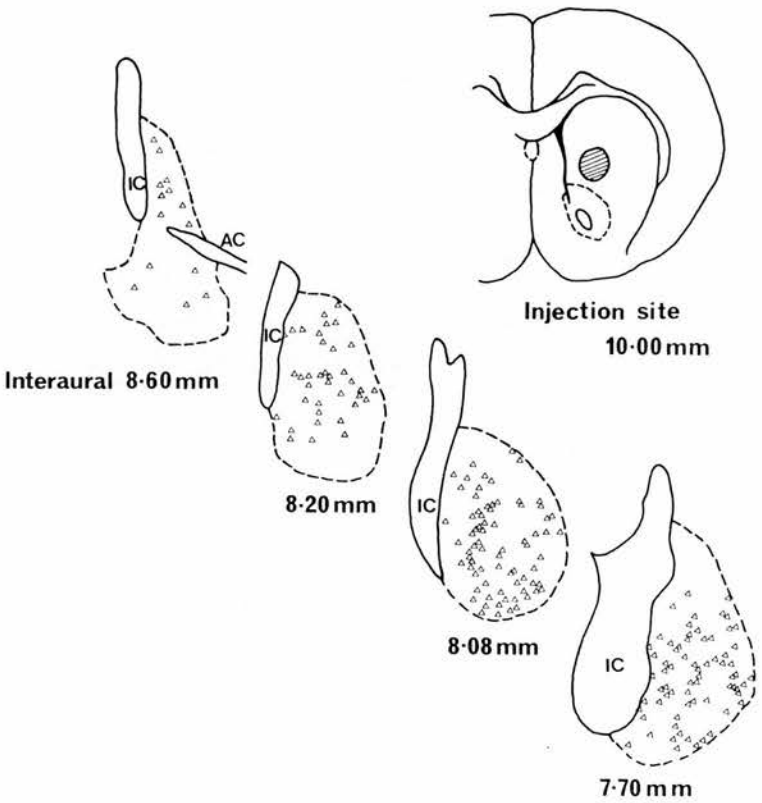

Fig. 5. A small deposit of True Blue at the site marked resulted in extensive labelling of neurones in the GP. The cell positions were traced from low power photographs of the distribution of the cells in sections of brain corresponding to the levels of the atlas illustrated. Only in the most anterior section are there cells outwith the boundaries of GP and the distribution is much more dorsal and lateral than that usually seen after cortical injections. Labels are as for earlier figures. AC – anterior commissure

antibody to ChAT. In this particular section, chosen to illustrate the largest overlap of retrogradely-labelled cells with ChAT positive cells, even some of the most dorsal neurones are double-labelled.

Discussion

The neurophysiological results suggest the existence of a pallidostriatal group of neurones, although none of the methods used distinguish between fibres ending in striatum and those passing through the nucleus.

The responses of the neurones to striatal stimulation – with the notable exception of antidromic activation – are similar to those seen intracellularly by other workers in rats (Park et al. 1982), cats (Levine et al. 1974) and monkeys (Yoshida et al. 1972). In the rat the corticofugal fibres run through the striatum and there are clear effects of cortical stimulation on pallidal neurones (Toan and Schultz 1985) so we cannot be certain that all the orthodromic activity we observed derives from striatum. Nevertheless, we found cells antidromically driven from both striatum and crus cerebri, which supports the anatomical demonstration by Staines and Fibiger (1984) that some of the neurones in GP project to both striatum and substantia nigra. The absence of a descending projection to subthalamus from small pallidocortical neurones reported in an anatomical study by van der Kooy and Kolb (1985) suggest that the neurones doubly antidromically driven are not these pallidocortical neurones. We cannot prove conclusively that the pallidostriatal cells are also pallidonigral, since the electrode for stimulation of the crus cerebri was situated immediately under the subthalamic nucleus. We may thus have seen activity due to stimulation of pallidosubthalamic axons (van der Kooy et al. 1981), although this could not have been a major source of error since we did not see orthodromic activation of the subthalamopallidal circuit similar to that reported by Perkins and Stone (1981).

The relatively low number of neurones showing antidromic activation from the striatum in this study is undoubtedly due to the topographic organisation which the pallidostriatal pathway shares with many other connections between the nuclei of the basal ganglia. Staines and Fibiger (1984) suggest that pallidostriatal, pallidonigral, and striatonigral pathways are in register, so that pallidal cells project to both origin and termination of a topographically designated group of striatonigral neurones. The striatopallidal fibres seem to innervate two anatomi-

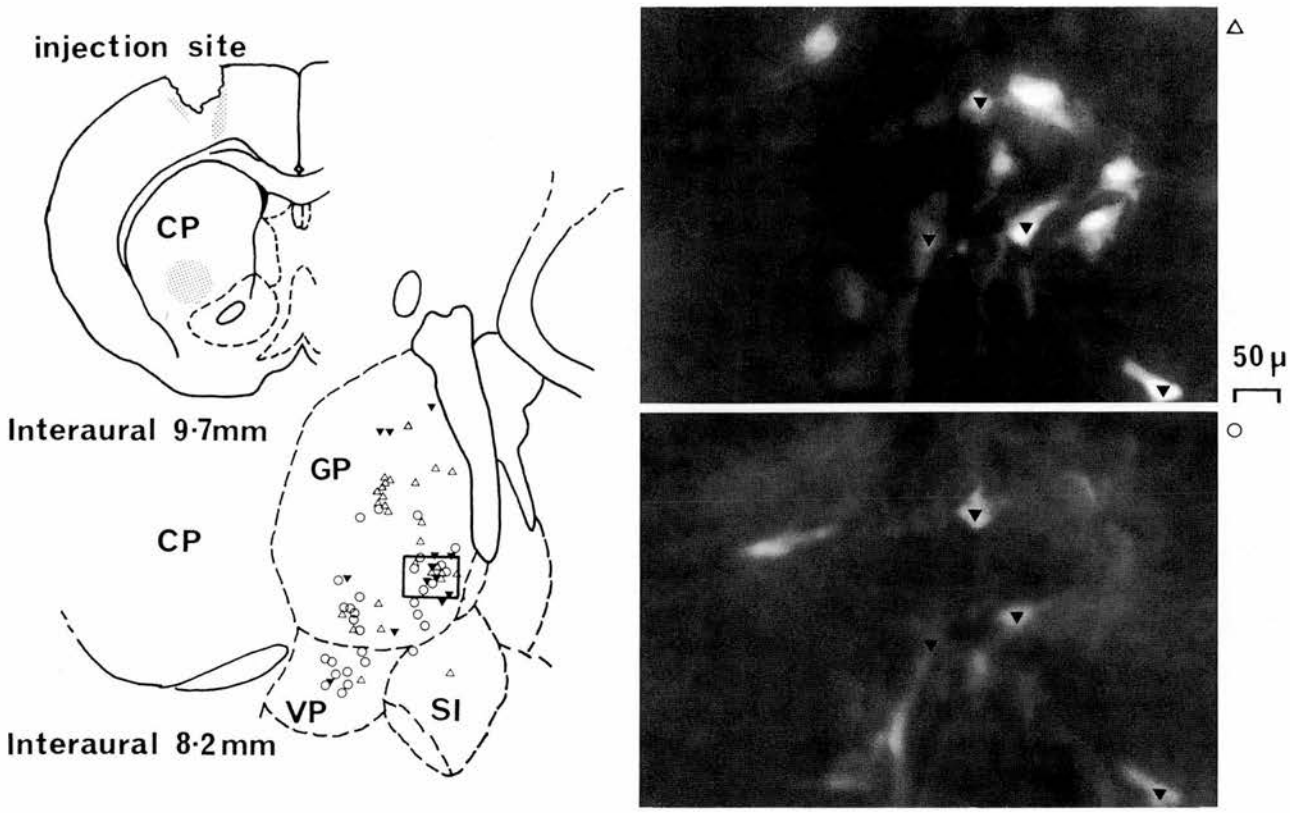

Fig. 6. This illustration comes from an animal in which the injection of fluorogold spread to include a considerable area of cortex (shaded area, beside the damage indicated). In this case there are cells double-labelled (▼) for both retrograde uptake of fluorogold (△) and also for the presence of ChAT (○), as well as many cells labelled with only one of the markers. The photographs are from the area of the inset rectangle in GP and illustrate the same section viewed under the filter set for fluorogold and that for ChAT. Double-labelled cells are indicated. Anatomical diagrams are annotated as before. SI – substantia innominata

cally distinct areas of the GP (Wilson and Phelan 1982), and it is as yet unclear how the pallidostriatal cells relate to either of these projections.

Only one third of the units anatomically localised to the GP met the criteria of firing rate (10–70 Hz) used by Walters and collaborators (1981, 1984). They used chloral hydrate anaesthesia or locally anaesthetised and paralysed rats, whereas all our animals were under halothane anaesthesia. Some of the cells we recorded may have been slowed, or silenced by the anaesthetic. However, a recent study by Toan and Schultz (1985) involved the use of chloral hydrate anaesthesia and it is clear from their figures that not all of their pallidal neurones were firing faster than 10 Hz. Neither group reports the silent cells which we saw activated by striatal stimulation, although an absence of spontaneous activity is included in the wide range of firing characteristics reported for pallidocortical (putative cholinergic) neurones (Aston-Jones et al. 1985; Reiner et al. 1987). Because in the rat these cells are not restricted to the ventral pallidal area (Fig. 6) the recording position alone cannot be used as a criterion to distinguish this group of cells from other pallidal neurones.

The neurochemistry of this output pathway of the GP is not yet known but the present data, along with previous investigations (Arbuthnott et al. 1983) suggest that the pallidostriatal cells do not contain substance P, enkephalin, or acetylcholine. Whatever their own transmitter, our results suggest that they respond to the lack of DA after a 6-OHDA lesion. Even though there is a higher proportion of cells firing faster, they are still within the normal range. Similar results are seen in the awake monkey after electrolytic lesions, or reserpine administration (Filion 1979), or after MPTP treatment (Filion et al. 1986, 1988; Miller and Delong 1987). The cells in our small sample are strikingly hyper-responsive to striatal stimulation even in anaesthetised animals (c.f. Filion 1986, 1988). The remarkable similarity between Fig. 4e and the illustration of the response of GP neurones to striatal stimulation in vitro

(Nakanishi et al. 1985) suggests that the 'hyper-responsive' neurones lack an important influence of dopamine which is also lost in vitro.

Our observations in rats which have had chronic lesions of the nigrostriatal dopaminergic pathway contrast with the acute responses of pallidal cells to intravenous application of dopamine agonists and antagonists (Bergstrom and Walters 1981; Bergstrom et al. 1984). In this situation dopaminergic activation increases firing rate or reduces the inhibitory action of GABA. One possible explanation is that during the six months since the 6-OHDA lesion long-term compensation for the loss of dopamine has occurred (Schultz and Ungerstedt 1978; Arbuthnott et al. 1987). Our higher average firing rate may suggest that this is the case, but until we have pharmacological data and a time course of the changes after 6-OHDA, it is too early to speculate.

We now have the baseline data from which to describe the effects of the loss of the dopamine input to striatum on these pallidostriatal cells which are themselves intimately connected with the output from striatum. The possibility of their making contact at both ends of the striatonigral pathway puts them in a very special position with respect to this important efferent pathway from the striatum. The evidence that striatonigral fibres carry the actions of dopamine relevant for behaviour (Garcia-Munoz et al. 1977; Arbuthnott and Wright 1982) makes these pallidal cells a prime target for modifications which may alleviate the consequences of dopamine depletion, as in Parkinson's disease.

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A NOVEL METHOD FOR TARGETING NEURONS IN A LIGHTLY FIXED STRIATAL SLICE PREPARATION.
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Intracellular filling of cells in the in vitro tissue slice provides detailed information about their dendritic fields. Methods have been developed for targeting specific cells by prelabeling them with transportable retrograde dyes or with nonspecific nuclear dyes such as 4,6-diamidino-2-phenylindole (DAPI). We here report a method for using a fluorescent Nissl stain (see Quinn and Weber, this meeting) that permits targeting of specific cell types according to the appearance of their perikarya, combined with injection of Lucifer Yellow, visible at the same wavelength. This method was developed in a lightly fixed striatal slice preparation. Slices 400µm thick were taken from perfused ferret brain and maintained in a bath on the stage of a microscope fitted with a 470nm epifluorescence filter. Meta-phenylene diamine was applied topically to produce a fluorescent Nissl stain. Cells with different perikaryal dimensions were targeted with a micropipette and Lucifer Yellow was injected under direct vision into the cell of choice. After a number of cells had been filled in a slice, it was post-fixed by immersion in 4% paraformaldehyde-phosphate buffer, and cut into 100µm thick sections which were then stained for cholinesterase. This method enables reconstruction of dendritic fields of striatal cells with respect to the histochemically definable striosomes, and should have broad applicability to the study of other regions of the central nervous system. *Supported by the Faculty of Medicine, Univ. of Edinburgh, the Whitaker Health Sciences Fund and NSF BNS 8720475.*

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INTRACELLULAR LABELLING OF MEDIUM SPINY NEURONS IN THE PRIMATE CAUDATE NUCLEUS: ANATOMICAL RELATIONSHIP OF DENDRITES TO STRIOSOMAL BORDERS

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With the technique of intracellular filling of cells in lightly fixed tissue slices, carried out in combination with staining for striosomes and immunochemistry specific to the intracellular dye, we investigated the orientations of dendrites of medium-sized spiny neurons lying near striosomes in the primate caudate nucleus. Slices 400um thick were cut from lightly fixed squirrel monkey brain. Cells were filled at random using a micropipette filled with an 8% solution of Lucifer Yellow (Sigma). Slices were post-fixed, sectioned at 40um, and stained for butyrylcholinesterase to demonstrate striosomes. Following this they were incubated overnight with an antibody to Lucifer Yellow (courtesy of Drs Kuwada and Knafl) and processed with diaminobenzidine. This sequence permitted the detailed morphology of the filled cells to be clearly distinguished under the light microscope in the presence of staining for the striosomal compartments. Of 206 filled medium-sized spiny neurons, 170 were in the matrix and 36 in striosomes. The dendrites of a number of neurons near striosomal borders, 17 in matrix, 15 in striosomes, avoided crossing distinct compartmental boundaries, whereas dendrites of 5 matrix cells and 4 striosomal cells clearly crossed between compartments. Thus, although many medium-sized projection neurons observe striosome/matrix borders, a subpopulation have the potential for integrating information from both compartments.

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